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VARIABILITY IN PHOMA LINGAM[1]

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INTRODUCTION

In the Puget Sound section of Washington a species of *Phoma* has been found to occur regularly as a minor leaf spot and stalk rot pathogen of seed plantings of cabbage (*Brassica oleracea* var. *capitata* L.) and Chinese cabbage (*B. pekinensis* (Lour.) Rupr.). On the latter host losses resulting from such infections have been important. The same fungus has also been found to cause mild infection on seed plants of turnip (*B. rapa* L.) and rutabaga (*B. napobrassica* (L.) Mill.).

The Puget Sound section has become this Nation's chief producer of cabbage seed primarily because seed grown there does not carry *Phoma lingam* Fr. (Desm.), the organism that causes blackleg, and *Xanthomonas campestris* (Pam.) Dows., the one that causes black rot. It became of interest, therefore, to determine the pathogenic capacity of these isolates of *Phoma* and to determine their relation both to the cabbage blackleg organism and to the organism of dry rot of rutabaga in Europe (*8*)[2] and New Zealand (*2*). This paper presents a description of the *Phoma* that occurs in the Puget Sound section and considers its variability in relation to that of *P. lingam* found associated with typical blackleg of cabbage in other sections of the United States.

REVIEW OF LITERATURE

In 1791 Tode (*9, p. 51 and pl. XVI, fig. 126*) described the blackleg organism of cabbage and named it *Sphaeria lingam*. Because he found the fungus occurring on dead cabbage stems, he considered it a saprophyte. In 1849 Desmazières (*3*), however, collected the same fungus on living plants and transferred it to the genus *Phoma*. In Denmark in 1894 Rostrup (*8*) described a disease of rutabaga that he attributed to an organism which he named *P. napobrassicae*. In 1918 Henderson (*5*) gave a detailed account of the occurrence of *P.*

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[2] Italic numbers in parentheses refer to Literature Cited, p. 132.

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lingam in Wisconsin, where it caused severe losses in cabbage. Henderson did not mention variability of the causal organism, but he described two kinds of pycnidia that it produced. He stated that considerable evidence existed for placing *P. napobrassicae* in the species *P. lingam*.

In 1905 Kirk (*7*) observed a dry rot of rutabaga in New Zealand and identified the causal organism as *Phoma napobrassicae* Rostrup. Cunningham (*2*) studied the growth rate, type, and pathogenicity of about 400 isolates from seed and other plant parts of rutabaga affected with dry rot. On the basis of growth rate and pathogenicity to nongrowing rutabaga roots he divided the isolates into 2 groups. Group 1 contained fast-growing, nonstaling forms which were weakly pathogenic; group 2 contained slow-growing, staling forms which were strongly pathogenic. Cunningham also studied cultures of *Phoma* obtained from cruciferous plants in Wisconsin, the Netherlands, and Africa and concluded that the dry rot in New Zealand, England, and continental Europe was caused by the same organism as blackleg of cabbage in the United States. He suggested *P. lingam* (Tode) Desm. as the correct binomial for the pathogen.

Cunningham's study of seed transmission of dry rot stimulated a reinvestigation of the disease in England and Ireland, since most of the seed planted in New Zealand was imported from those countries. In 1933 Hughes (*6*) essentially confirmed Cunningham's findings of strain-group relations as far as growth rate was concerned, but he was not able to confirm his separations on the basis of pathogenicity. He suggested the existence of one strain of *Phoma lingam* that occurred primarily on rutabaga in Europe and New Zealand and of another that was associated with cabbage and various other crucifers, but not with rutabaga. Hughes presented limited evidence to support this idea. He concluded further that the strain associated with rutabaga was identical with the American blackleg organism and that both belonged to the species *P. lingam*.

In 1934 Buddin (*1*) reported the results of his reinvestigation of dry rot in England. He found that five of the seven representative isolates sent him by Cunningham did not conform to *Phoma lingam* and were weakly pathogenic. In fact, he identified some of them as belonging to other genera of fungi. He was able to show, however, by single-spore cultures that considerable variability existed within *P. lingam*, largely in rates of growth and amounts of staling. He interpreted his observations to mean that the isolates comprised a graded series rather than clear-cut groups.

Grove (*4, v. 1, p. 70*) reduced Rostrup's species to varietal status as *Phoma lingam napobrassicae* (Rostr.) Grove.

From this discussion it is evident that, according to the International Rules of Botanical Nomenclature, the cabbage blackleg organism would be cited as *Phoma lingam* (Fr.) Desm., since Tode is pre-Friesian.

MATERIALS AND METHODS

The sources of all cultures studied are given in table 1.

Single-spore cultures were obtained by pouring a very dilute conidial suspension from the mass cultures over the surface of water agar. After incubation for 24 to 36 hours the individual germinating spores

were lifted with a needle under a stereoscopic microscope and each was planted in a culture tube containing suitable medium. The following system of culture designations was used: A letter, a group of letters, or a word followed only by a number represented a monoconidial isolate from one of the original mass cultures listed in table 1 (for example, W11 and PS40). Sectors arising from such single-spore cultures were indicated by placing after the designation of the single-spore culture the letter M and a number to indicate the order in which the sector arose. Thus, W11M1 was the first mycelial sector from W11. Culture W11M2M1 was the first mycelial sector of the second mycelial sector from W11. The letters SS and a number following a culture designation signified single-spore transfers of the isolate in question (for example, S26SS1).

All spore measurements were made under an oil-immersion lens at 900 magnifications. The spores were taken from a heavily sporulating culture on potato-dextrose agar and were mounted in glycerol jelly.

All isolates that were studied comparatively were grown on media from the same source.

TABLE 1.—*Sources of all Phoma isolates studied*

Culture or disease designation	State of origin	Contributor	Host	Key to single-spore isolates
Phoma lingam	Wisconsin	J. C. Walker	Cabbage	W1 to W200.
Cabbage blackleg	Iowa	W. J. Hooker	do	Iowa-1 to Iowa-30.
Do	New York	C. Chupp	do	NY1 to NY28.
Do	Wisconsin	R. H. Larson	do	W300 to W550.
Phoma sp	Oregon	I. H. Vogel	do	S1 to S50.
Do	Washington	G. S. Pound	Chinese cabbage	PS1 to PS150.
Do	do	do	Cabbage	PS200.
Do	California	K. Baker	Sweet alyssum	C1 to C25.

EXPERIMENTAL RESULTS

ISOLATION OF STRAINS

From a mass culture of *Phoma* from a Chinese cabbage plant in the Puget Sound section, 150 monoconidial isolates were established on potato-dextrose agar. As no variability was noted among these isolates, one, PS40, was selected for further study. A second monoconidial culture, PS200, obtained from a mass culture from a cabbage plant infected by *Phoma* in the Puget Sound section was also studied in some detail since it differed slightly from PS40. A third culture, C1, of this group, which differed only slightly from both PS40 and PS200, was sent to the writer from California by Kenneth Baker. It was originally isolated from a seed field of *Lobularia maritima* (L.) Desv.

Two hundred monoconidial isolates were established from a culture of *Phoma lingam* received from J. C. Walker, of the University of Wisconsin. These were designated as W1 to W200. From these isolates 2 distinct culture types were obtained on potato-dextrose agar. Six (represented herein by W43) of the 200 isolates were fast-growing cultures which produced an abundance of aerial mycelium and relatively few pycnidia. The remaining cultures (represented herein by W11) were all very slow in growth rate and produced an abundance of pycnidia, but very little aerial mycelium.

In February 1944 I. H. Vogel, of the Associated Seed Growers, Inc., gave the writer a leaf with a single *Phoma* lesion, suspected to be blackleg, from a cabbage plant growing at Salem, Oreg. Fifty monoconidial isolates (designated S1 to S50) were made from the original culture obtained from the lesion. Three distinct culture types were found among these monoconidial isolates. One type (represented by S1) was very similar to the slow-growing type obtained from the Wisconsin culture. A second type (represented by S39) differed from the S1 type only in the production of small, black, sclerotiumlike bodies on the potato-dextrose agar. The third type (represented by S26) was a fast-growing culture, which differed from the fast-growing isolates of the Wisconsin culture in that it produced fewer pycnidia and a greenish-black pigment in the mycelium.

Cultures obtained from blackleg material supplied from Iowa and New York were alike and practically identical with the fast-growing isolates obtained from the Wisconsin culture. Monoconidial isolates Iowa–11 and NY1 were selected to represent the cultures from Iowa and New York, respectively. From cabbage blackleg material received from Wisconsin in 1945, 250 single-spore isolates were established. All of these isolates (W300 to W550) were essentially alike and practically identical with the cultures from New York and Iowa and with the fast-growing isolates obtained from the previous culture from Wisconsin. Thus, the following arbitrary groups and subgroups of *Phoma*, according to their characteristics on potato-dextrose agar, were obtained from cultures isolated from cruciferous plants.

Group 1:
- A. Slow-growing isolates of Wisconsin culture. (W11.)
- B. Slow-growing isolates of Oregon culture that produced no sclerotiumlike bodies. (S1.)
- C. Slow-growing isolates of Oregon culture that produced sclerotiumlike bodies. (S39.)

Group 2:
- A. Fast-growing isolates of Wisconsin cultures, as well as cultures from Iowa and New York. (W43, NY1, Iowa–11, and W307.)
- B. Fast-growing isolates of Oregon culture. (S26.)

Group 3: Puget Sound isolates and the isolate from California. (PS40, PS200, and C1.)

CULTURAL CHARACTERS OF STRAINS

Growth Type

Examination of table 2 will reveal that the various representative isolates had distinct cultural characters. Isolates PS40, PS200, and C1 were distinct from all others in the production of a yellow to brown, water-soluble pigment in the medium. Mycelial growth was much coarser than that of other isolates, and a yellow to tannish-brown pigmentation of mycelium was very distinct. Sporulation was moderate. The pycnidia of PS40 and C1 usually occurred in clusters, but occasionally developed singly.

Isalates W43, NY1, Iowa–11, and W307 showed only minor differences in type of growth. All produced an abundance of white to gray or grayish-brown aerial mycelium which ultimately became grayish black. No staling occurred at room temperature. Sporulation was slight and pycnidia developed singly.

Isolate S26 was similar in many respects to NY1 and Iowa–11, but it was distinct on potato-dextrose agar in the production of a mycelium that had a powdery sheen and later developed a characteristic greenish-black pigmentation from the center of the colony out. Sporulation was very slight; pycnidia developed singly and were irregularly scattered over the agar surface.

TABLE 2.—*Characteristics of representative Phoma isolates grown on malt-extract, oatmeal, and potato-dextrose agars for 6 weeks at room temperature*

Isolate	Malt-extract agar	Oatmeal agar	Potato-dextrose agar
W11	Growth slow; staling ultimately complete; mat outline very irregular with dendritic pattern during first 2 weeks, becoming rather regular later, but always less circular than those of other isolates. Aerial mycelium fairly abundant, white at periphery of mat, greenish black at center. Pycnidia very abundant, small, black. Black pigment beneath mycelial mat with age.	Growth moderately fast; staling slight; mat outline regular. Aerial mycelium scant (much less than on malt-extract agar), grayish white, in somewhat concentric rings, giving a zonate appearance. Pycnidia very abundant, scattered evenly over surface, small, black. Slight black pigment beneath mycelial mat with age.	Growth fairly slow; staling moderate; mat outline regular to very irregular, often with dendritic pattern. Aerial mycelium scant, grayish white; submerged mycelium milky white. Pycnidia very abundant, scattered evenly over surface and submerged, small, black. Slight black pigment beneath mycelial mat with age.
S1	Growth slow; staling ultimately complete; mat outline always very regular. Aerial mycelium scant but more than on other agars. Pycnidia very abundant, scattered evenly over surface, small, black. Zonation distinct with dark center, grayish-brown midzone, and white periphery. Differing from W11 chiefly in conspicuous zonation and regularity of mat outline.	Growth moderately fast; staling slight; mat outline very regular. Aerial mycelium very scant. Pycnidia very abundant, scattered evenly over surface at first, but in concentric rings later, small, black.	Growth moderately fast at first; staling complete after 3 weeks (ultimately distinctly more than that of W11); mat outline very regular. Aerial mycelium very scant. Pycnidia abundant, scattered evenly over surface and submerged, small, black. Slight zonation of alternating light and dark areas very pronounced; black pigment beneath mycelial mat; very different from that of W11 with age.
S39	Almost same as S1 except for more conspicuous zonation.	Growth moderately fast; staling slight; mat outline regular. Aerial mycelium scant. Pycnidia very abundant, very conspicuously, concentrically zonate (very different from W11 and S1). Resembling S1 in other respects.	Growth moderately fast at first; staling complete with age; mat outline very feathery. Pycnidia scant. Sclerotiumlike bodies scattered over surface, but piling up in center, about 0.5 mm. in diameter, black. Differing from growth of S39 on other agars and differing distinctly from all other isolates in production of sclerotiumlike bodies. Pigment beneath mycelial mat very pronounced, black with age.
S26	Growth moderately fast (much slower than on potato-dextrose agar); staling very little; mat outline very regular. Aerial mycelium abundant, cottony, elevated in center, later becoming dirty white, tending somewhat to collapse and form concentric rings. Pycnidia scant, covered by mycelium. Black pigment developing beneath fungus mat from center out.	Growth very fast; staling none; mat outline regular. Aerial mycelium moderately abundant, white at first, but becoming yellowish white, dense in center producing a bull's-eye effect. Pycnidia very scarce. Slight pinkish pigment developing in center.	Growth very fast; staling very little; mat outline very regular. Aerial mycelium moderately abundant, coarse, powdery white at first, becoming greenish black from center out until whole mat involved. Pycnidia scant, relatively large. Differing from other isolates in greenish-black pigment and powdery sheen of mycelium.

TABLE 2.—*Characteristics of representative Phoma isolates grown on malt-extract, oatmeal, and potato-dextrose agars for 6 weeks at room temperature*—Con.

Isolate	Malt-extract agar	Oatmeal agar	Potato-dextrose agar
Iowa-11	Growth moderately fast; staling none; mat outline very regular. Aerial mycelium abundant, elevated in center, white. Pycnidia scant. Black pigment developing beneath mycelial mat. Resembling S26.	Growth very fast; staling none; mat outline regular. Aerial mycelium moderately abundant (much less than on malt-extract agar). Pycnidia scant. Pigment developing slowly beneath mycelial mat, black.	Growth very fast; staling none; mat outline very regular. Aerial mycelium moderately abundant, white to gray. Pycnidia at center scant, large; pycnidia later scattered throughout, small. Black pigment developing beneath mycelial mat; agar occasionally pinkish. Differing from W11 and S1 in type of growth and amount of sporulation; resembling S26 in many respects.
NY1	Same as Iowa-11 except for much flatter mat center.	Same as Iowa-11 except for less abundant mycelial growth and greater zonation with alternate rings of gray and tan-white mycelium.	Same as Iowa-11 except for being zonate at the periphery of mat.
W43	Almost same as Iowa-11; resembling NY1 except for elevated center like that of Iowa-11.	Almost same as Iowa-11.	Almost same as Iowa-11 and NY1 except for dense greenish-black pigmentation of central zone, which becomes studded with pycnidia more quickly than Iowa-11 and NY1.
W307	Not studied.	Not studied.	Very similar to Iowa-11, differing in that mycelium is grayish brown and sometimes sulfur yellow in central zone.
PS40	Growth moderately fast; staling none; mat outline very regular. Aerial mycelium very abundant, very coarse, white at first, but rapidly turning yellow to tan to brown from center out and remaining much darker at center. Pycnidia scant to moderately abundant. Yellow to brown pigment diffused throughout agar.	Growth very fast; staling none; mat outline very regular. Aerial mycelium very abundant, coarse, yellow brown. Pycnidia moderately abundant, usually in clusters rather than evenly scattered over surface. Conspicuous pigment developing throughout, pink at first, becoming yellowish brown.	Growth very fast; staling none; mat outline regular. Aerial mycelium abundant, very coarse, white at first, but changing to yellow to yellowish brown, becoming much darker in center and producing a bull's-eye effect. Pycnidia like those developed on oatmeal agar; also strictly aerial pycnidia in old cultures, abundant, black. Yellow pigment diffused throughout agar.
PS200	Same as PS40 except for noticeably lighter center of mat and finer mycelium.	Not studied.	Same as PS40 except for aerial mycelium, white at first and becoming less brown and more zonate, and pycnidia more abundant and in concentric zones.
C1	Not studied.	do	Very similar to PS200; cottony white mycelium, becoming more grayish brown than tannish brown. Pycnidia clustered in conspicuous zonate rings.

Isolate W11 was distinct from all others, but it was closest to S1. On potato-dextrose agar it produced very little aerial mycelium, but rather a milky-white submerged growth. Its growth outline was mostly irregular and often assumed a markedly dendritic pattern. Sporulation was much more profuse than that of the fast-growing cultures. Pycnidia were closely arranged with no semblance of concentric zonation. Ultimately cultures became staled and dark. The growth outline of isolate S1 was regular in contrast to the dendritic pattern often produced by W11, and a greater tendency for zonation

existed; ultimately staling was more pronounced and cultures assumed a distinctly blacker appearance.

Isolate S39 differed from S1 on potato-dextrose agar in the production of numerous black sclerotiumlike bodies (about 0.5 mm. in diameter) instead of small pycnidia. Pycnidia later developed in these sclerotiumlike bodies, and spore masses were discharged. Spore production, however, was much less than that of S1. A few small pycnidia like those of S1 were occasionally produced along with the sclerotiumlike bodies. On malt-extract and oatmeal agars, however, these sclerotiumlike bodies were not produced; instead, a profuse development of small, black pycnidia occurred and the growth of isolate S39 was practically identical with that of S1.

The cultural characteristics of representative isolates at room temperature are shown in figure 1.

Growth type at 30° C. was different from that at room temperature for most of the isolates studied. Isolate PS40 produced a much more intense pigmentation of agar, the pigment ultimately becoming plum red. The mycelium was coarser and much darker than at room temperature. Isolates Iowa–11 and NY1 produced white to grayish-white mycelial mats with less mycelium and almost none of the pigmentation found at room temperature. Isolate S26 produced an elevated mycelial mat with considerable grayish-black aerial mycelium and a hard stromalike growth beneath the mat at 30°. Both W11 and S1 were regular in outline at 30° and produced a raised growth with considerable aerial mycelium, gray in W11 and grayish black in S1. A hard stromalike growth developed beneath the mat, and pycnidia were fewer than at room temperature.

Growth Rate

Tables 3 and 4 show that isolates W11, S1, and S39 were very similar in growth rate at room temperature but that minor differences occurred. Staling of S1 was more pronounced and its ultimate growth was less than that of W11. Regardless of the type of medium used W11, S1, and S39 always grew slowly and eventually staled. Frequently fan-shaped growth developed from portions of a staled culture, giving it a very irregular outline. These areas of new growth were often numerous enough to coalesce and the result was a considerable increase in growth diameter. Isolates S26, W43, NY1, Iowa–11, and PS40 were all very similar in growth rate, and staling rarely occurred at room temperature. The growth outline was always regular, regardless of type of medium used. Isolate W43 showed a considerably slower growth on rutabaga-dextrose agar than did others of this group. Isolates C1 and W307 were not included in these comparative tests.

In table 5 comparative growth rates at room temperature and 30° C. for some of the isolates are given. At 30° isolates W11 and S1 were very similar, both being much more severely staled than at room temperature. S26, a fast grower at room temperature, was severely staled at 30° and ultimately its growth was no greater than that of S1. Isolates NY1 and Iowa–11 showed no appreciable difference in growth at the two temperatures until after 8 to 10 days, when growth at the high temperature became less than at room temper-

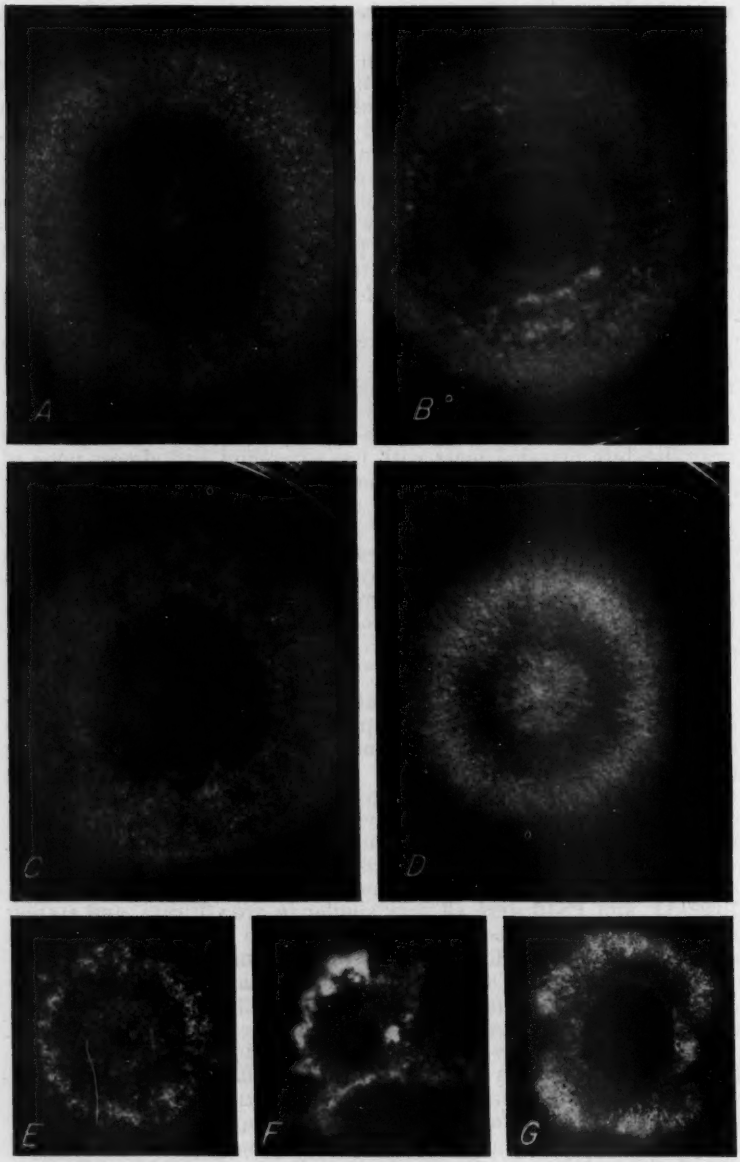

FIGURE 1.—Representative 2-week-old cultures of *Phoma* on potato-dextrose agar at room temperature: *A*, Iowa–11; *B*, PS40; *C*, W43; *D*, S26; *E*, S1; *F*, W11; *G*, S39.

ature. Isolate NY1 was staled more than Iowa–11; it was ultimately completely staled. Growth of both isolates at 30° fell considerably short of that at room temperature. Isolate PS40 had approximately the same growth rates at the two temperatures.

TABLE 3.—*Growth rates of representative Phoma isolates in 6-ounce bottles, at room temperature, on malt-extract and potato-dextrose agars*

[Each value is an average from 3 single-spore cultures]

Medium and isolate	Growth in indicated period (days)							
	4	7	11	13	15	18	20	40
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
Malt-extract agar:								
W11	2	7	10	12	15	22	26	73
S1	2	7	12	14	18	26	29	71
S39	2	7	12	14	19	25	30	69
S26	3	8	18	24	31	37	43	101
W43	4	11	20	27	39	48	55	(1)
NY1	4	12	24	37	47	57	65	(1)
Iowa-11	4	14	26	38	47	57	64	(1)
PS40	5	18	27	37	49	56	63	(1)
Potato-dextrose agar:								
W11	2	9	30	40	52	59	66	92
S1	2	14	32	38	45	51	53	60
S39	2	11	32	39	49	56	63	76
S26	6	28	50	65	75	87	93	(1)
W43	8	28	48	61	81	88	(1)	(1)
NY1	8	33	58	74	88	(1)	(1)	(1)
Iowa-11	8	32	58	73	88	(1)	(1)	(1)
PS40	11	35	57	73	84	(1)	(1)	(1)

[1] Growth had filled bottle and was beyond measurement.

TABLE 4.—*Growth rates of representative Phoma isolates in 6-ounce bottles, at room temperature, on rutabaga-dextrose agar*

[Each value is an average from 3 cultures inoculated with 3-mm. agar disks from 10-day-old cultures]

Isolate	Growth in indicated period (days)									
	1	3	5	7	9	11	13	15	17	21
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
W11	5	10	16	25	34	37	42	47	51	57
S1	5	14	22	29	36	38	41	43	44	50
S39	7	18	28	36	40	42	43	45	46	47
S26	8	21	33	45	60	73	81	88	97	(1)
W43	5	11	22	35	47	54	58	63	69	76
NY1	7	20	33	48	65	79	89	102	(1)	(1)
Iowa-11	8	22	35	49	65	81	92	104	(1)	(1)
PS40	7	19	31	45	60	74	83	95	(1)	(1)
PS200	6	19	31	44	60	75	87	99	(1)	(1)

[1] Growth had filled bottle and was beyond measurement.

STABILITY OF STRAINS

Numerous mycelial transfers and single-spore subcultures were made of the isolates being studied. Several saltant strains were isolated from the single-spore cultures. These saltant strains were just as stable through successive transfers as the parent strains. Mutants arising as mycelial sectors occurred most frequently in cultures subject to staling. This was especially true of isolates W11

TABLE 5.—*Growth rates of representative Phoma isolates in 6-ounce bottles, at room temperature and 30° C., on potato-dextrose agar*

[Each value is an average of 8 hypha-tip cultures of each monoconidial isolate]

Isolate	Temperature (° C.)	Growth in indicated period (days)										
		6	8	10	12	14	16	18	22	27	33	51
		Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
W11	Room	6	16	28	39	51	61	69	85	97	104	(1)
	30°	4	8	10	13	16	19	21	25	27	30	34
S1	Room	10	22	31	39	44	50	54	63	72	79	(1)
	30°	3	6	8	10	14	19	23	27	31	35	41
S26	Room	22	39	53	67	82	96	(1)	(1)	(1)	(1)	(1)
	30°	5	7	8	10	13	16	18	21	24	29	39
NY1	Room	23	39	53	66	80	91	98	(1)	(1)	(1)	(1)
	30°	24	39	46	50	53	54	57	60	65	68	77
Iowa-11	Room	25	42	57	71	84	97	(1)	(1)	(1)	(1)	(1)
	30°	27	45	58	67	74	77	80	83	87	94	(1)
PS40	Room	25	40	52	65	80	93	(1)	(1)	(1)	(1)	(1)
	30°	30	48	62	73	87	97	(1)	(1)	(1)	(1)	(1)

[1] Growth had filled bottle and was beyond measurement.

and S1 growing at 30° C. Practically always the saltants grew faster than the parent strains. Growth characters on potato-dextrose agar of some of the saltant strains are as follows:

W11M1.—Fast-growing, white sector that arose at 30° C. Outline of mycelial mat regular but feathery; aerial mycelium abundant, white, with conspicuous cottony balls; pigment beneath mat slight, pinkish; at room temperature sporulation moderate and pycnidia very large, angular, and compound.

W11M2.—Fast-growing sector that arose at 30° C. At room temperature outline of mycelial mat regular; pycnidia abundant, small, black; growth resembling that of W11. At 30° growth faster; pycnidia moderately abundant, small, black; pigment beneath mat greenish black; growth very different from that of W11.

W11M3.—Fast-growing, white sector that arose at room temperature. Mycelium white, mostly submerged; pycnidia abundant, small, with thin, light-colored walls; spore discharge heavy, giving entire surface a bright-pink color; distinctly different from other isolates.

W11M4.—Fast-growing, white sector that arose at 30° C. Pycnidia scant, small, black; differing from W11M1 in almost complete absence of sporulation at room temperature.

W33M2.—Fast-growing, white sector that arose from W33, a sister culture identical with W11, at room temperature; almost identical with W43 and Iowa-11.

W33M3.—Yellow-tan sector that arose at room temperature. Mycelium almost entirely submerged; pycnidia none; sclerotiumlike bodies yellow, 0.25 to 0.5 mm. in diameter; medium pigment yellow.

W33M5.—Fast-growing, white sector that arose at room temperature; identical with W33M2.

S1M1.—Fast-growing sector that arose from staled culture at room temperature; identical with S26.

S1M1M1.—Fast-growing, fleshlike, white sector. Mycelial mat dense, white, ultimately pinkish; sporulation and pigmentation slight. Growth of this type in many S cultures, often overrunning cultures that were untouched for several months.

S1M3.—Fast-growing, white sector that arose in staled culture at 30° C. Growth rates same at 30° and room temperature; aerial mycelium pinkish white; sporulation none; with age very similar to S1M1M1.

S1M4.—Fast-growing, white sector that arose at 30° C. Mycelium with cottony balls like that of W11M1; pycnidia very large, compound, like those of W11M1; very distinct from all other S cultures.

S39M1.—Sector that arose in staled culture at 30° C.; mycelium abundant; pycnidia none.

S26M1.—Fast-growing, white sector that arose in staled culture at 30° C.; mycelial pigment yellow green.

S26SS1.—One of 10 single-spore transfers; identical with S1.

S6M1.—Sector that arose as a fleshlike, white growth in S6, a sister culture identical with S1, at room temperature; identical with S1M3.

S6M1M1.—Sector that arose at room temperature; identical with S26.

Iowa-11M1.—Sector that arose at room temperature; mycelium abundant; pycnidia none; pigment none.

Iowa–11SS1.—One of 10 single-spore transfers, indistinguishable from W11 at room temperature, but with 3 times as much growth at 30° C.

Other saltations that occurred are not listed here. The fleshlike types were recovered particularly from the S isolates. Of the original 200 single-spore isolates of the W series more than 60 changed from the slow-growing, small-pycnidiate type to the fast-growing, large-pycnidiate Iowa–11 type during a 7-month incubation in test tubes without transfer. One changed until it was identical with W11M3; 1 was identical with W33M3; 2 were identical with W11M4; and others showed intermediate rates of growth and degrees of sporulation. Very similar changes occurred in the S series during the 7-month period; many of the S1 type changed to faster growing forms. One recovered was identical with S1M3; 1 was identical with S1M4; and several were of the S26 type. These data seem to indicate that slow-growing forms frequently change to fast-growing types when conditions for growth are unfavorable. During the 7 months without transfer the cultures had dried down considerably.

It may be noted that some of the different groups set up on page 116 have been found to be linked together by saltation. Thus, the fast-growing type of group 2 A was derived from the slow-growing type of group 1 A in W33M2 and W33M5. Similarly, group 2 B was derived from group 1 B in S1M1 and S6M1M1. Also group 1 A was derived from group 2 A in Iowa–11SS1, and group 1 B was derived from group 2 B in S26SS1. In addition, several distinct forms such as W11M1, W11M3, S39M1, and Iowa–11M1 were isolated.

No sectoring was ever observed in isolate PS40.

MORPHOLOGY OF STRAINS

In table 6 are given the sizes of conidia of various representative isolates. It can be seen that there was relatively little difference in the widths but that differences did occur in lengths. The isolates obtained from the East (W11, Iowa–11, and NY1) did not differ much in spore size; nor did the isolates of the S series (S1, S26, and S39). However, conidia of the S group were noticeably shorter than those of the eastern group. Conidia of isolate PS40 were still shorter, but their lengths were nearer those of the S isolates. The close agreement of the sizes of the conidia of the S isolates might suggest their origin to be somewhat removed from that of the isolates from the East.

The sizes and the types of the pycnidia are given in table 7. The isolates were not grouped for pycnidial size as they were for spore size. A great diversity of pycnidial size and shape occurred among the isolates. Although pycnidia of a single isolate varied considerably in size and shape, they were regular enough to be characteristic; W11 and S1 always produced small pycnidia, and Iowa–11 and NY1

always produced large ones. When a slow-growing, small-pycnidiate form gave rise to a fast-growing type, there was a corresponding change in the size of the pycnidia (for example, W33M2, W33M5, and S1M1).

TABLE 6.—*Measurements of conidia of representative Phoma isolates grown for 2 weeks on potato-dextrose agar*

[Measurements of 100 spores except as indicated]

Isolate	Width			Length		
	Average	Maximum	Minimum	Average	Maximum	Minimum
	μ	μ	μ	μ	μ	μ
W11 [1]	1.69	2.50	1.08	4.34	5.83	3.25
Iowa-11	1.67	2.08	1.08	4.39	5.83	3.33
NY1 [2]	1.73	2.33	1.17	4.41	5.83	3.33
S1	1.66	2.08	1.25	4.08	5.50	3.35
S39	1.62	2.33	1.00	4.07	5.87	3.25
S26	1.66	2.50	1.25	4.09	5.91	3.17
PS40	1.62	2.08	1.25	3.97	5.80	3.08

[1] 150 spores measured.
[2] 50 spores measured.

TABLE 7.—*Sizes and types of pycnidia of various Phoma isolates and their saltants grown on potato-dextrose agar at room temperature*

[Measurements and observations on 100 pycnidia of each isolate]

Isolate	Average width	Description of pycnidial type
	μ	
W11	179	Size and shape rather regular (round to broadly flask-shaped with broad base; slightly beaked and slightly papillate); usually simple, but sometimes compound; decidedly black; spore masses readily discharged.
W11M1	650	Regular and roundish at first, but becoming irregular and angular with numerous fingerlike protuberances thought to be small pycnidia; spores not readily discharged, but large masses released by crushing.
W11M3	156	Shape very similar to that of W11, but size noticeably smaller and wall color noticeably lighter; pycnidia made pinkish by spore masses even before discharge; spore masses discharged profusely.
S1	139	Size and shape similar to those of W11 except smaller and slightly more flask-shaped.
S1M4	744	Indistinguishable from W11M1.
S26	236	Size rather irregular (some flask-shaped like those of S1; others roundish like those of Iowa-11); lighter colored than those of S1; spore discharge slight.
S39	633	Sclerotiumlike bodies large, roundish, smooth, hard, black, with slight spore discharge at different points, probably indicating presence of pycnidia that developed peripherally in them.
Iowa-11	385	Size and shape in general rather regular (large, roundish); occasionally beaked, irregular, and compound; lighter in color and more papillate than those of W11; spore masses readily discharged.
W43	332	Almost same as Iowa-11.
PS40	340	Size and shape very irregular (some very small, flask-shaped like those of W11 others large, round like those of Iowa-11, distinctly beaked); many simple, others compound; black.

PATHOGENICITY OF STRAINS

Numerous tests were made to determine the pathogenicity of representative isolates and their saltants. One method of inoculation was to soak seeds of the host species being tested in a spore suspension for 48 hours before planting. Within 2 weeks the emerged seedlings developed cotyledonary lesions and severe damping-off. Isolates PS40, PS200, and C1 were easily distinguishable from all others by this method. The cotyledonary lesions caused by these isolates

appeared 3 to 5 days earlier than those caused by other isolates and were of a distinctly different type; usually they were marginal and chocolate brown from the beginning and enlarged slowly; the pycnidia produced on the lesions were few, relatively large, and more brown than black. Cotyledonary lesions produced by all other isolates were almost identical and appeared as shrunken, dark-green areas, which rapidly enlarged and became thickly studded with pycnidia before the lesion surfaces became dark. Results obtained on radish (*Raphanus sativus* L.), Chinese cabbage, cabbage, turnip, rutabaga, kale (*Brassica oleracea* var. *acephala* DC.), and brussels sprouts (*B. oleracea* var. *gemmifera* Zenker) in one such test are given in table 8. It is apparent that isolates S1, S39, and S26 were generally less virulent than the others.

TABLE 8.—*Results of inoculating seeds of various cultivated crucifers with representative isolates of Phoma*

Isolate	Approximate portion of seedlings of indicated species killed						
	Radish	Chinese cabbage	Cabbage	Turnip	Rutabaga	Kale	Brussels sprouts
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
W11	40	80	80	60	100	100	100
S1	40	40	60	40	100	60	40
S39	20	40	40	20	60	60	40
S26	20	20	20	20	40	40	40
NY1	80	100	100	40	100	100	80
W43	60	80	80	60	100	100	100
PS40	80	100	100	40	60	100	100

Inoculations of older plants were made by spraying the leaf surfaces with spore suspensions and by sponging leaves previously sprinkled with powdered carborundum with a ball of absorbent cotton dipped in a spore suspension. The carborundum removed some of the bloom and thus increased the wetting of the leaves. Stem inoculations were made by dipping roots of small seedlings in a spore suspension before transplanting and by pouring spore and mycelial suspensions on the surfaces of soil in which plants were growing, with and without wounding the stems. In table 9 are given the results obtained with large plants of cauliflower (*Brassica oleracea* var. *botrytis* L.), broccoli (*B. oleracea* var. *botrytis* L.), rape (*B. napus* L.), and other species.

On leaves of brussels sprouts isolates S1, S26, S39, and PS40 produced very similar effects. Black necrotic flecks surrounded by a conspicuous chlorotic halo developed at the infection sites. These flecks enlarged very slowly and rarely exceeded 5 mm. in diameter, but occasionally they developed into extensive necrotic areas. Sporulation was slight. Isolates W11, Iowa–11, W43, and NY1 produced much more extensive necrosis than the other group, causing complete blighting of the leaves. Lesions did not develop the conspicuous halo produced by the other strains. Sporulation was slight. On stems of brussels sprouts the isolates differed from each other only in virulence and in degree of sporulation. All produced lesions typical of blackleg, but none was as virulent as on cabbage or cauliflower.

TABLE 9.—*Results of inoculating leaves and stems of various cultivated crucifers with representative Phoma isolates and their saltants*

[+, slight virulence; ++, moderate; +++, severe; ++++, very severe]

Isolate	Virulence of symptoms on host indicated											
	Cabbage		Brussels sprouts		Cauliflower		Broccoli leaves	Turnip leaves	Rutabaga leaves	Kale leaves	Chinese cabbage leaves	Rape leaves
	Leaves	Stems	Leaves	Stems	Leaves	Stems						
W11	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
W11M1	++	------	++	++	++	++	+++	------	------	++	------	------
W11M2	+++	------	+++	++	+++	+++	++++	------	------	+++	------	------
W11M3	+	------	+	+	+	+	++	------	------	+	------	------
W11M4	++	------	++	+++	+	++	+++	------	------	+	------	------
S1	+++	+++	++	++	++	++	++	++	++++	+	++++	++
S1M1	------	------	------	++	++	++	++	------	------	+	------	------
S1M3	------	------	------	+	+	+	------	------	------	------	------	------
S1M4	------	------	------	++	+	++	++	------	------	+	------	------
S26	+++	+++	++	++	++	++	------	+	+++	------	++++	++
S26M1	------	------	------	++	------	------	------	------	------	------	------	------
S39	+++	+++	++	++	++	++	------	+	++++	------	+++	++
S39 M1	------	------	------	+	------	------	------	------	------	------	------	------
Iowa–11	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
W43	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
NY1	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
W307	------	++++	------	------	+++	+++	++++	------	------	+++	------	------
PS40	+++	++	++	+++	++	++	++	------	++	++	+++	+
C1	+++	++	------	+++	++	++	------	+	++	------	------	------

On leaves of cabbage, cauliflower, and broccoli symptoms produced by PS40 were distinct from all others except those produced by W11M3, which they closely resembled. Numerous black necrotic flecks developed in 6 to 10 days. These small lesions enlarged very little before the leaves became chlorotic and abscised. Sporulation was scant to none. Occasionally a few lesions enlarged and developed light centers and pycnidia as in nature. Several weeks were required to reach this stage. If the spore load used was not very light, the leaves fell off before this stage of development was reached. On cabbage and cauliflower isolate C1 was identical with PS40. Isolates W11, W11M1, W11M2, NY1, Iowa–11, W43, and W307 produced identical symptoms as circular lesions which rapidly enlarged, coalesced, and caused complete necrosis. Affected tissue collapsed as if killed by steam and was covered with pycnidia while still dark green. Eventually the lesion surfaces became ashen gray. Isolates S1, S26, S39, S1M1, and S1M4 produced similar symptoms but were generally less virulent than the eastern isolates. Isolate W11M3 was very much less virulent than W11 and produced symptoms more like those of PS40. Typical symptoms produced on broccoli leaves by isolates W11, PS40, and W11M3 are shown in figure 2.

On the stems of large cabbage plants (fig. 3) all the isolates tested produced lesions typical of blackleg. Again PS40 and the S isolates were less virulent than W11, NY1, and Iowa–11. Isolates of the second group often killed the plants.

On leaves of Chinese cabbage isolate PS40 was distinct from all others tested (fig. 4). Numerous black flecks appeared 2 to 4 days before symptoms were produced by other isolates. These angular lesions developed very slowly and reached a diameter of not more than 3 to 5 mm. before the leaves became yellow and abscised.

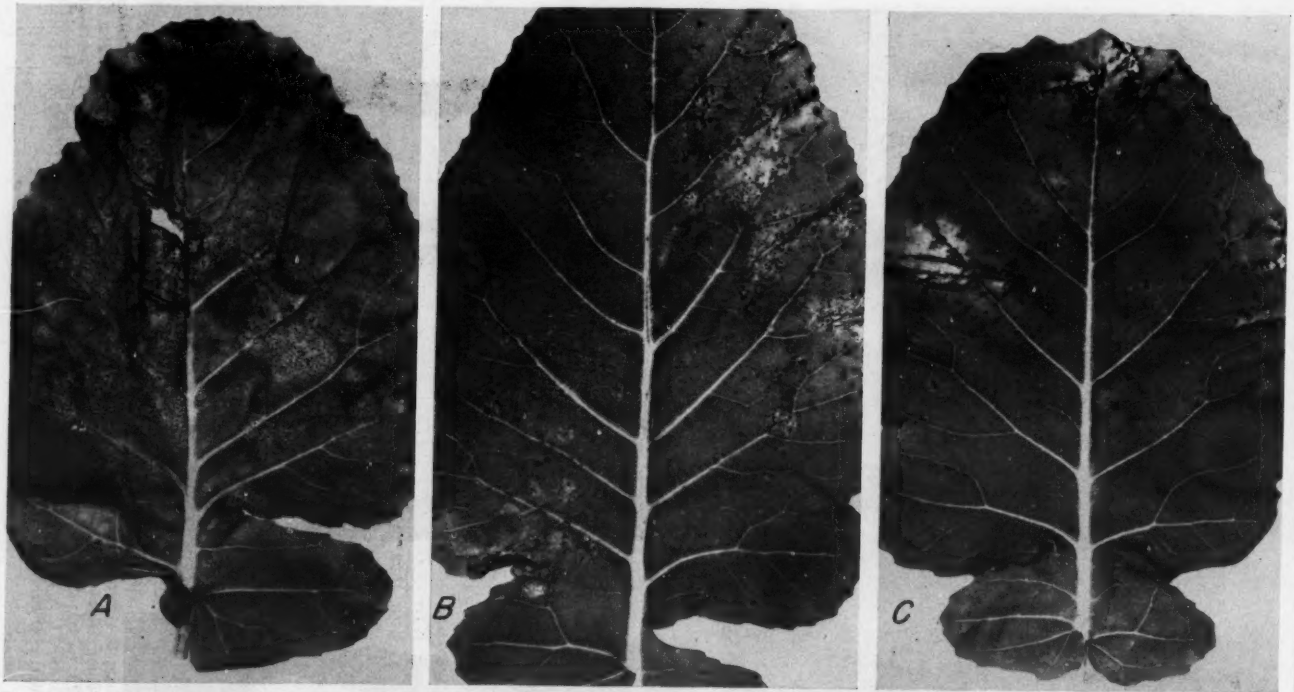

FIGURE 2.—Symptoms produced on broccoli leaves by three *Phoma* isolates: *A*, W11; *B*, PS40; *C*, W11M3. Note abundance of pycnidia on lesions produced by W11 and their absence on others. Note also the similarity of the lesions produced by PS40 and W11M3.

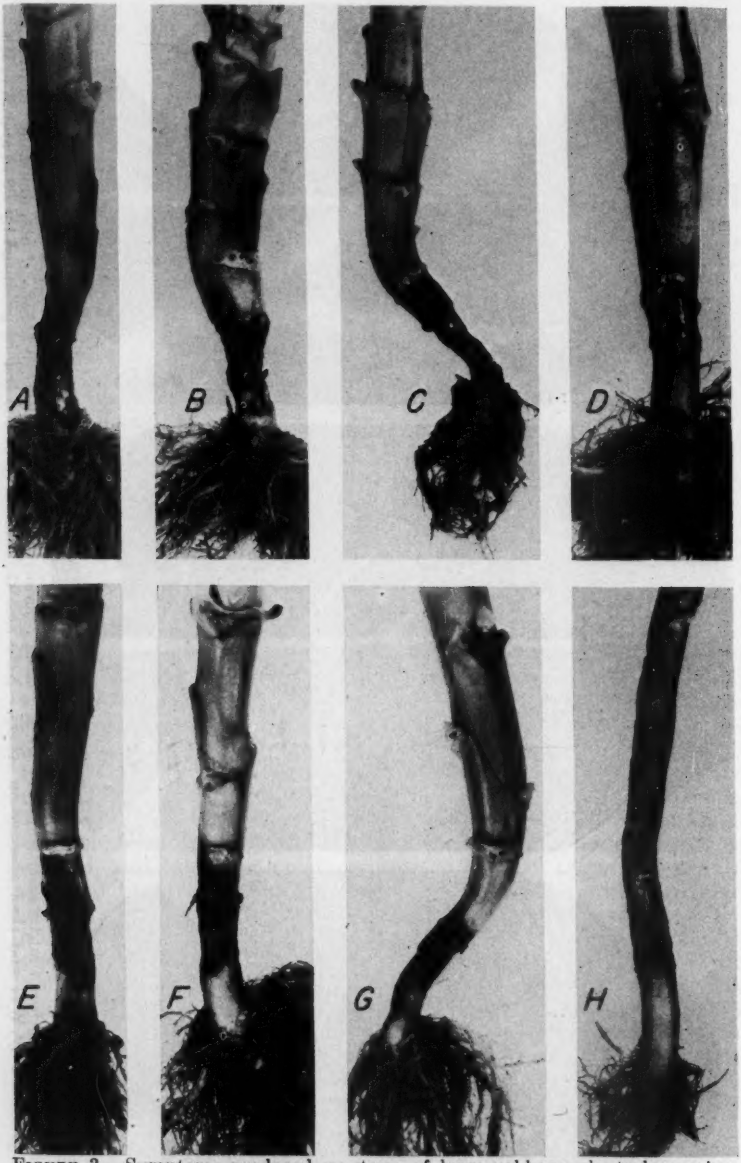

FIGURE 3.—Symptoms produced on stems of large cabbage plants by various *Phoma* isolates: *A*, Iowa–11; *B*, NY1; *C*, W11; *D*, uninoculated; *E*, S26; *F*, S39; *G*, S1; *H*, PS40. Note that the lesions produced by the eastern isolates (Iowa–11, NY1, and W11) are more severe than those produced by the western isolates (S26, S39, S1, and PS40).

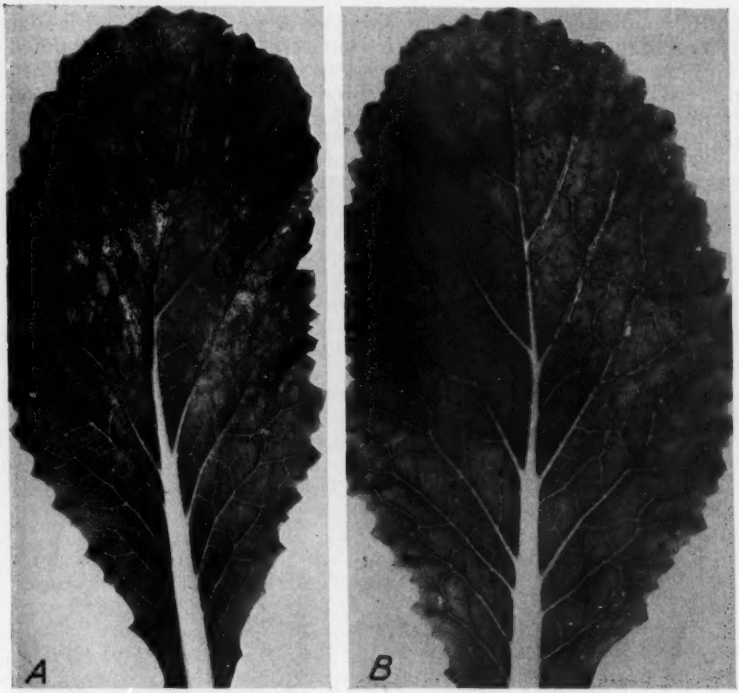

FIGURE 4.—Symptoms produced on Chinese cabbage leaves by *Phoma* isolates: *A*, Iowa–11; and *B*, PS40. Note abundance of pycnidia on lesions produced by Iowa–11 and the flecking only produced by PS40.

Sporulation was scant. When a very light spore load was used, however, lesions reached diameters of 10 to 20 mm., became brown, and were covered with pycnidia. When the infections occurred on the fleshy petiole, necrosis often extended to the leaf axil and lesions formed on the stems. These symptoms approximated those occurring in nature. Isolates S1, S26, S39, W11, NY1, and Iowa–11 caused small flecks, which rapidly enlarged and became covered with pycnidia before extensive necrosis was evident. There was little difference in the symptoms produced by these isolates.

On turnip leaves all isolates produced only pin-point necrotic flecks. These lesions did not develop beyond the fleck stage, and no abscission resulted. No isolate sporulated on this host. On rutabaga, however, all isolates produced extensive infection and brown necrotic lesions up to 20 mm. in diameter. All isolates produced similar effects on this host, but PS40 and C1 were less virulent than the others.

In the spring of 1945 rutabaga plants in midbloom were sprayed with spore suspensions of S1, S26, S39, W11, NY1, Iowa–11, and PS40. At biweekly intervals during the remainder of the blossoming period the plants were watered with a hose nozzle to simulate rain-

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storm conditions. At maturity the pods were examined and lesions typical of those caused by *Phoma lingam* were found on pods inoculated with all isolates (fig. 5). The lesions produced by isolate PS40 differed from the others only in producing fewer pycnidia. Seed from infected pods was accidentally destroyed before a test for seed transmission could be made.

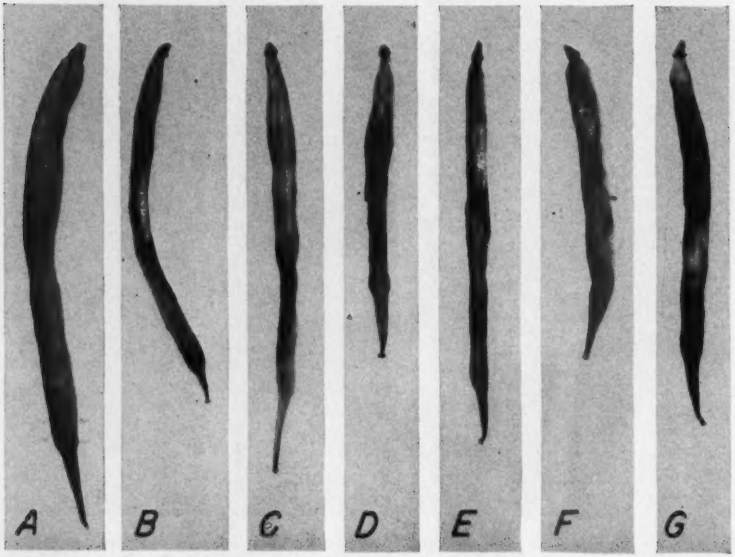

FIGURE 5.—*A*, Healthy rutabaga seed pod. *B–G*, Lesions produced on rutabaga seed pods by various *Phoma* isolates: *B*, S1; *C*, S26; *D*, S39; *E*, W11; *F*, NY1; *G*, PS40. Note similarity of the lesions.

Thus, in the pathogenicity tests recorded isolates of the S series and PS40 were nearly always found to be less virulent than isolates W11, NY1, and Iowa–11. Isolate W11M3, a saltant of W11, was much less virulent than W11 and more like PS40 in the type of symptoms that it produced.

DISCUSSION

The disease caused by a *Phoma* species described in this paper has been observed to occur regularly in the seed-growing district of the Puget Sound section of Washington. Only traces have been found in cabbage fields, where it caused scattered leaf lesions and occasional stalk rots of seed plants. Typical blackleg of cabbage, however, has not been observed in this district. The same species of *Phoma* has also been observed to infect rutabaga, turnip, and Chinese cabbage seed plants in the Puget Sound section. On Chinese cabbage it has been more virulent than on other hosts. On this host lesions commonly occur on leaf petioles from which they spread to the leaf axil, and stalk infections result. As flower heads emerge from leaf axils,

they become infected and severe blighting of florets results. Lesions on stalks at the ground level and above are common and cause stalks to break over. Sporulation on lesion surfaces is abundant. Lesions have not been found to occur naturally on seed pods, and it is believed that the dry season during which pods develop and mature checks the spread to seed pods. That the Puget Sound *Phoma* can infect pods was shown by experimental work. Although in the threshing process seed surfaces could come in contact with spores, it is not believed that this would result in much seed transmission, since naked spores do not remain viable long. It was not determined whether this strain infected seeds internally as does the *Phoma* that causes cabbage blackleg.

How the Puget Sound *Phoma* lives over from season to season was not determined; but, since there is neither much crop rotation nor any crucifer-free period in the production of cruciferous seed crops in the Puget Sound section, overwintering would not be a factor in the disease cycle. The rather weak appearance of this fungus on cabbage in nature plus its widespread occurrence might indicate that it occurred commonly as a soil-borne saprophyte and less commonly as a parasite. Its extreme pathogenicity on certain hosts in the greenhouse would indicate, however, that it is not necessarily a facultative parasite. This is further borne out by its virulence on Chinese cabbage in nature. In view of the long and intensive culture of crucifer seed crops in the Puget Sound section, it seems more probable that this *Phoma* is carried from crop to crop by plant debris and living hosts.

The *Phoma lingam* that causes typical blackleg of cabbage was shown to be very variable, and several cultural strains of it were isolated. These strains differed markedly in growth type and rate and slightly in pathogenicity and morphology. Saltation occurred frequently and was found to link the strain groups together. Unfavorable growing conditions apparently increased the frequency of saltation. It is very probable that further study would reveal numerous other types. In view of the wide variability of *P. lingam* it seems logical to conclude that the Puget Sound isolates, the Oregon isolates, and the California isolate, which fall within that range of variability in morphology, physiology, and pathogenicity, are variants of *P. lingam*. Although the organism causing dry rot of rutabaga in New Zealand and Europe was not examined by the writer, it seems logical to conclude that it also belongs to *P. lingam* since the results obtained by Cunningham (*2*), Hughes (*6*), and Buddin (*1*) indicate that it falls within the range of variability of this species.

It may be well to point out, however, that, although the isolates of *Phoma* from the Puget Sound section were within the range of variation of *P. lingam*, they showed certain points of difference. They, with the isolate from California, were distinct from all other isolates in the production of a water-soluble, yellow or brown pigment in the media, in a yellow to tannish-brown pigmentation of mycelium, and in the coarser appearance of the mycelium in culture. The Puget Sound isolate PS40 was apparently stable in culture, whereas isolates from the East and from Oregon were more or less variable. The conidia of PS40 were shorter than the spores from any other isolate.

Isolates from the Puget Sound section and from California were also distinct from other isolates in the type of lesion produced on cotyledons of seedlings grown from inoculated seeds and in the symptoms produced on inoculated leaves of older cabbage plants.

In view of these findings it is still a question whether the strain of *Phoma* found in the Puget Sound section would cause typical blackleg on cabbage in sections favorable to the disease even if it were transmitted there by seed from the Puget Sound section. Although it may be regarded as a strain of *P. lingam*, its potential danger as a harmful seed-borne pathogen on cabbage has yet to be demonstrated. Even though nothing in this study indicates that cabbage seed grown in the Puget Sound section is not free from blackleg, it should be pointed out that the Pacific coast seed-producing section cannot be regarded as free from all strains of *P. lingam*. Moreover, the occurrence of a mild strain of what appears to be *P. lingam* in the important Puget Sound section and of other strains in the Willamette Valley of Oregon is reason for emphasizing that the possibility of seed contamination and transmission in that section should not be overlooked.

SUMMARY

A *Phoma* has been found to occur regularly as a minor leaf spot and stalk rot pathogen of seed plants of cabbage, Chinese cabbage, rutabaga, and turnip in the Puget Sound section of Washington. Experiments showed that it is widely pathogenic on other cruciferous plants. It produces typical blackleg symptoms on *Brassica oleracea* in greenhouse tests.

In determining the relation of this fungus to *Phoma lingam*, the cabbage blackleg organism, a survey of variability in the latter was made. Several cultural strains that differed in type and rate of growth, degree of staling, and amount of sporulation were established by single-spore technique. In a comparative study of these strains numerous saltants that tended to link the strains together were isolated. Cultural studies of the variant strains indicated that the Puget Sound isolates differed from those from typical blackleg material in absence of staling, in production of water-soluble yellow to brown pigment in both medium and mycelium, and in a coarser mycelium.

The Puget Sound isolates produced symptoms that differed from those produced by isolates from typical blackleg material in type and virulence and were thus separable from the latter.

Only slight differences in conidial size were found among the strains, but marked differences in size and shape of pycnidia occurred.

The Puget Sound strain of *Phoma* appeared to fall within the range of variability in morphology, physiology, and pathogenicity of *P. lingam* and is considered a variant of it.

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ELIMINATION AND RETENTION OF POLLEN STERILITY IN POTATO IMPROVEMENT [1]

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INTRODUCTION

Improvement in the potato (*Solanum tuberosum* L.) has generally been accomplished through sexual breeding. Since pollen sterility interferes with sexual breeding, potato improvement would be facilitated if this factor were eliminated from the breeding material. Pollen sterility is, however, a desirable character in a potato variety. Complete pollen sterility when present prevents fruiting in this normally self-pollinated crop. Bartholdi (*2*)[2] secured a significant increase in yield of tubers by removing flower buds from a self-fruitful (fertile-pollen) variety, and a significant decrease in yield by pollinating a nonfruiting (sterile-pollen) variety and producing fruit development. Thus pollen fertility is essential for potato improvement but undesirable in a variety intended for cultivation. More complete information concerning the inheritance of pollen sterility would aid in the formulation of a breeding procedure that would reduce the interference from pollen sterility to a minimum and yet provide a means for its retention and utilization.

Pollen sterility in potato varieties may be complete (sterile-pollen varieties) or partial (fertile-pollen varieties). The fertile-pollen varieties can be further classified according to the percentage of pollen that is stainable with acetocarmine (*9*). A description of the breeding behavior of a number of sterile-pollen and fertile-pollen varieties selected from the breeding material of the Minnesota Agricultural Experiment Station will be presented, together with a discussion of the significance of the results in respect to breeding procedure for the improvement of the potato.

CHARACTERISTICS OF STERILE-POLLEN AND FERTILE-POLLEN PLANTS

The breeding behavior of varieties was analyzed with respect to pollen sterility by classifying their progenies into sterile-pollen and fertile-pollen plants.

Fertile-pollen plants were characterized by abundant pollen, with the stainable grains invariably round and plump, 35 to 50 microns in size, and the nonstainable grains uniformly smaller and resembling collapsed spheres, and by the absence of pollen grains of the type

[1] Received for publication March 11, 1946.
[2] Italic numbers in parentheses refer to Literature Cited, p. 145.

indicative of irregular meiosis. Examples of pollen from fertile-pollen plants are shown in figure 1.

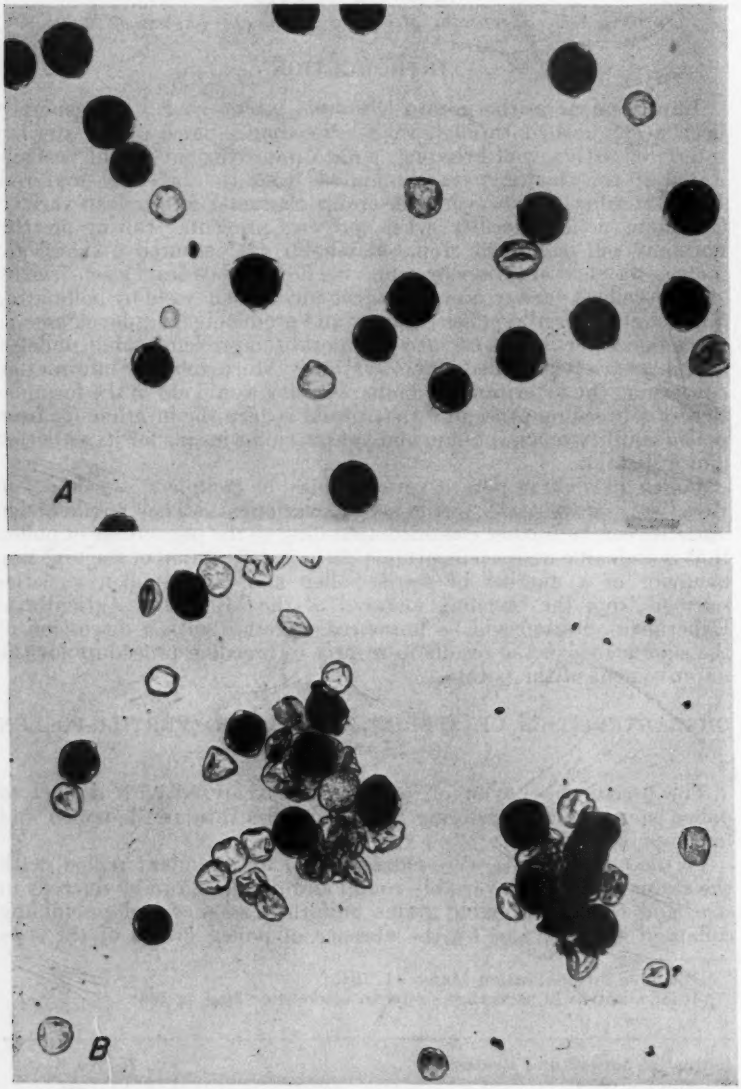

FIGURE 1.—Stainable and nonstainable pollen grains from (*A*) the fertile-pollen clone 15–2, which had 76.9 percent of stainable pollen, and (*B*) from the fertile-pollen clone 13–1, which had 24.9 percent of stainable pollen. ×365.

Sterile-pollen plants were characterized by the absence of normal appearing stainable pollen grains (fig. 2) and by the presence of pollen indicative of irregular meiosis. The pollen of sterile-pollen plants ranged in quantity from scant to abundant; the grains ranged in size from one-fourth to twice the size of normal stainable grains, and were irregularly shaped (fig. 2). Further indications of irregular meiosis

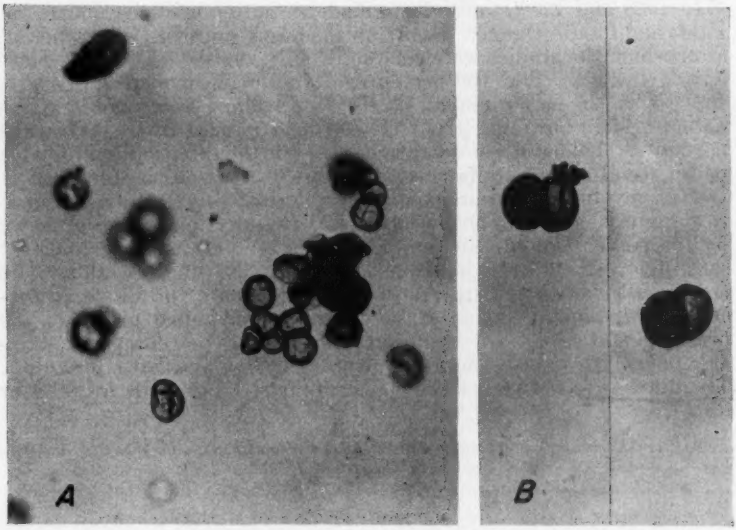

FIGURE 2.—*A*, Pollen from the sterile-pollen clone 77–9 having 5.6 percent stained pollen; *B*, pollen from the sterile-pollen clone 77–7, having 2.8 percent stainable pollen. X365.

were the presence of large grains with thick rugose walls of the sort described by Fukuda (*6*) as having been dreived from unreduced pollen-mother cells. Grains having two or more distinct sections or cells (fig. 2) were present at times. Three-, four-, and five-sectioned grains were less common.

Occasionally in sterile-pollen plants a few grains and one or more sections of a multisectioned grain were stainable, but these could be distinguished from normal stainable grains by various irregularities in shape and size. Scantiness of pollen was associated with a reduction in size of both single and multisectioned grains. A few plants contained neither stainable pollen grains nor abnormalities indicative of irregular meiosis.

The complete pollen sterility of sterile-pollen plants is, according to Arnason (*1*) and Longley and Clark (*10*), the result of the failure of normal microspore formation during meiosis. This view is supported by numerous meiotic irregularities observed in sterile-pollen plants (*1*, *3*, *5*, *6*, *7*, *10*). The more common meiotic irregularities observed were: (1) Failure of the chromosomes to pair; (2) lagging of the chromosomes on the spindle; and (3) failure to complete the normal reduction-division process.

According to Arnason (*1*) and Longley and Clark (*10*), the partial sterility of the fertile-pollen plants is conditioned, after what appears to be a normal meiosis, by microspore abortion, which frequently occurs after the spores have reached normal pollen-grain size.

SAMPLING THE MATERIAL

In 1938 and 1939 studies were made at both University Farm (St. Paul) and Castle Danger, Minn., on the north shore of Lake Superior, after which the studies were conducted only at Castle Danger. Since it is well known that the percentage of stainable pollen varies with changes in the environment (*4*, *9*), the studies were made only in periods where flowering and fruit setting appeared to be proceeding normally. Under these conditions, very little variation in stainable pollen resulting from differences in season or location was obtained.

The percentage of stainable pollen was relatively uniform on identical material grown in both years and at two locations. This is shown by the percentage of stainable pollen observed in four selections for each of 2 years and two locations (table 1). The four selections were chosen because they were known to approximate the range in percentage of stainable pollen found in the fertile-pollen plants in the breeding material. No significant difference in percentage of stainable pollen due to either location or years, or both, is shown in table 1. The differences between the clones were fairly consistent for all four tests.

TABLE 1.—*Percentage of stainable pollen in 4 fertile-pollen parent clones at University Farm and Castle Danger in 1938 and 1939*

Parent clones	Stainable pollen in clones at indicated locations and seasons				Mean [1]
	1938		1939		
	University Farm	Castle Danger	University Farm	Castle Danger	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
15-2	76.1	83.0	78.7	74.3	78.5
5-1	67.2	76.0	75.3	73.3	73.0
12-7	57.2	50.9	60.3	34.7	50.8
13-1	29.2	26.5	20.6	23.5	25.0
Mean [1]	57.4	59.6	58.7	51.5	

[1] Mean difference necessary for significance, 9.44 percent.

The mean percentages of stainable pollen obtained in sexual progenies at University Farm and at Castle Danger are presented in table 2. Four selfed and three crossed seedling progenies were used in this study. It will be noted that there was no significant difference due to location in the mean percentage of stainable pollen. These results suggest that limiting the studies to periods favorable to blooming tended to make the data obtained in different seasons and at different locations fairly comparable. Since a careful study of the data failed to indicate any influence of season or location on percentage of stainable pollen, these factors have been omitted in the presentation of later data.

BREEDING BEHAVIOR OF PLANTS

RECIPROCAL CROSSES

In the present study five sets of reciprocal crosses were made between parents which differed significantly in percentage of stainable pollen. The number of sterile-pollen and fertile-pollen plants and the means in percentage of stainable pollen in the fertile-pollen portion of the progenies of the reciprocal cross are given in table 3. In all five cases, the two progenies of reciprocal crosses differ significantly in the proportion of sterile-pollen to fertile-pollen plants and in the mean percentage of stainable pollen in the fertile pollen-plants. The differences between the two progenies of reciprocal crosses is associated with a similar difference in the stainable pollen of the female parents.

TABLE 2.—*Mean percentages of stainable pollen in the fertile-pollen plants in duplicate samples of selfed and crossed progenies at University Farm and Castle Danger in 1939*

Progenies	Stainable pollen in progenies [1] at—		Progenies	Stainable pollen in progenies [1] at—	
	University Farm	Castle Danger		University Farm	Castle Danger
	Percent	*Percent*		*Percent*	*Percent*
13–1 selfed	46.5±9.7	39.7±4.1	75–5×15–2	67.4±4.0	61.3±8.5
5–1 selfed	32.7±6.0	30.2±7.3	15–2×5–1	68.1±2.1	66.2±4.8
75–5 selfed	43.8±4.3	50.4±11.7			
15–2 selfed	66.3±3.0	56.4±6.3	Mean	49.4	47.9
13–1×5–1	20.9±6.0	31.3±10.7			

[1] In fertile-pollen plants.

TABLE 3.—*Sterile-pollen and fertile-pollen plants obtained in 5 reciprocal crosses between parents differing in percentage of stainable pollen*

Parents	Stainable pollen in female parent	Sterile-pollen plants	Fertile-pollen plants	Stainable pollen in progeny
	Percent	*Number*	*Number*	*Percent*
13–1 × 15–2	24.9	28	39	20.7
15–2 × 13–1	76.9	5	40	33.6
12–7 × 5–29	56.3	41	10	8.5
5–29 × 12–7	71.8	5	48	48.6
17–2 × 11–1	32.8	30	70	32.5
11–1 × 17–2	90.0	1	150	73.9
80–7 × 15–2	56.4	7	9	23.6
15–2 × 80–7	76.9	0	12	61.3
106–55 × 5–2	26.1	62	50	22.6
5–2 × 106–55	74.7	12	106	53.2

In all five reciprocal crosses female parents with a relatively low percentage of stainable pollen gave relatively low proportions of fertile-pollen plants as compared with female parents that had a relatively high percentage of stainable pollen. It is evident from the results of the five reciprocal crosses that parents with a low percentage of stainable pollen transmit more pollen sterility as female parents than as male parents. These results are in harmony with those of Salaman and Lesley (*11*), who found that the two progenies of a reciprocal cross were distinctly different in respect to pollen sterility and in fruit setting.

BREEDING BEHAVIOR OF PLANTS WHEN SELFED AND WHEN USED AS FEMALE PARENTS

The behavior of reciprocal crosses suggested that the factors responsible for sterile-pollen plants tended to be eliminated in the aborted pollen. Thus, with the reduced transmission of these factors through the pollen, the character of the progeny with respect to pollen sterility would be mostly determined by the breeding behavior of the female parent. If this were true, one might expect only slight differences in pollen sterility between the progenies of an individual when selfed and when used as a female parent in crosses irrespective of the male parent.

In table 4 are given the number of sterile-pollen and fertile-pollen plants in both the crossed and selfed progenies of nine female parents. For five of these nine, the crossed and selfed progenies do not vary widely from each other. The crossed progeny from 80–7 has a slightly higher proportion of sterile-pollen plants than its selfed progeny. The crossed progenies from 12–7, 21–2, and 13–1 have a much higher proportion of sterile-pollen plants than their selfed progenies. An association between the amount of stainable pollen in the parent and the proportion of fertile-pollen plants in the progeny is indicated, except for the crossed progenies of parents 21–2 and 12–7. Thus, for two of the nine parents, the breeding behavior in crosses did not seem to be closely related to the behavior when selfed or to the stainable pollen in the parent. This fact suggests that in some crosses the male parent may have a significant influence on the pollen sterility of the progeny.

CROSSES SHOWING THE RELATIVE INFLUENCE OF MALE AND FEMALE PARENTS

The possible influence of the male parent was studied by comparing crossed progenies having the same female parent but different male parents. The results obtained by crossing each of eight female parents with a number of different male parents are presented in table 5. In general, the progenies within the groups having the same female parent tend to be alike in the proportion of sterile-pollen to fertile-pollen plants. Nevertheless, there are some differences between the progenies of a group that are worth noting. In table 5, for example, the crossed progenies from male parents 12–7 and 80–7 had a higher proportion of fertile- to sterile-pollen plants than the progenies from the other male parents in the same group, and, with one exception, these other male parents had a higher percentage of stainable pollen. The difference between the male and female breeding behavior of selections 12–7 and 80–7 was also apparent when reciprocal crosses were made and highly contrasting progenies were obtained (table 3).

As indicated by the data in tables 3–5, the percentage of stainable pollen in a clone and its breeding behavior on selfing and as a female parent in crosses was not related to its breeding behavior when used as a male parent. The difference between the male and female breeding behavior of selections 12–7 and 80–7 might indicate that the factors for the relatively high proportion of sterile-pollen plants in their crossed progenies when used as female parents were not transmitted through the pollen of selections 11–25, 15–2, and 75. This explanation assumes that there was some transmission of the factors responsible for sterile-pollen plants through the pollen of some

TABLE 4.—*Sterile-pollen and fertile-pollen plants obtained in the crossed and selfed progenies of 9 female parents with different percentages of stainable pollen*

Female parents	Stainable pollen in parent	Type of progeny	Sterile-pollen plants	Fertile-pollen plants
	Percent		*Number*	*Number*
11–1	90.0	Crossed	0	151
		Selfed	0	38
15–2	76.9	Crossed	6	107
		Selfed	1	43
75–5	72.9	Crossed	0	30
		Selfed	5	43
5–29	71.8	Crossed	5	48
		Selfed	0	7
80–7	56.4	Crossed	13	15
		Selfed	6	13
12–7	56.3	Crossed	41	10
		Selfed	6	11
21–2	49.1	Crossed	43	8
		Selfed	5	10
17–2	32.8	Crossed	30	79
		Selfed	8	19
13–1	24.9	Crossed	57	81
		Selfed	6	12

TABLE 5.—*Sterile-pollen and fertile-pollen plants in crossed progenies having the same female parent but different male parents*

Cross	Stainable pollen in—		Sterile-pollen plants	Fertile-pollen plants
	Female parent	Male parent		
	Percent	*Percent*	*Number*	*Number*
15–2 × 5–1	76.9	69.5	1	55
15–2 × 80–7	76.9	56.4	0	12
15–2 × 13–1	76.9	24.9	5	40
80–7 × 15–2	56.4	76.9	7	9
80–7 × 5–1	56.4	69.5	6	6
13–1 × 15–2	24.9	76.9	28	39
13–1 × 5–1	24.9	69.5	29	24
13–1 × 80–7	24.9	56.4	0	18
77–9 × 75–5	5.6	72.9	18	4
77–9 × 11–25	5.6	72.4	117	3
77–9 × 5–29	5.6	71.8	58	15
77–9 × 12–7	5.6	56.3	26	11
76–1 × 75–5	4.3	72.9	18	4
76–1 × 11–25	4.3	72.4	56	1
76–1 × 5–29	4.3	71.8	26	8
76–1 × 12–7	4.3	56.3	13	6
77–7 × 75–5	2.8	72.9	34	3
77–7 × 11–25	2.8	72.4	52	5
77–7 × 5–29	2.8	71.8	32	3
77–7 × 12–7	2.8	56.3	68	31
77–8 × 75–5	0	72.9	31	0
77–8 × 11–25	0	72.4	23	1
77–8 × 5–29	0	71.8	36	0
77–8 × 12–7	0	56.3	30	3
Russet Rural × 15–2	0	76.9	60	13
Russet Rural × 11–25	0	72.4	93	14
Russet Rural × 5–29	0	71.8	109	4

selections. To the breeder, the significant aspect of the breeding behavior of the pollen parents shown in table 5 is that fertile-pollen plants such as 12–7, 13–1, and 80–7 whose percentage of stainable

pollen is low and whose progenies when selfed and when used as female parents in crosses may give a relatively high proportion of sterile-pollen plants, may transmit less sterility through the pollen than male parents having a significantly higher percentage of stainable pollen.

The influence of the male parent on the crossed progeny is, however, relatively small as compared with that of the female parent. The influence of the female parents is shown in table 6, which gives the segregation obtained from different female parents crossed with the same male parent. The largest differences in segregation between comparable progenies of female parents was obtained in groups 1, 4, and 5. These groups contained female parents with a wider range of percentage of stainable pollen than the other groups. An association between the percentage of stainable pollen in the female parent and the proportion of sterile- to fertile-pollen plants in their progenies is indicated in group 1 and to a lesser extent in groups 4 and 5. Some female parents (12–7 and 77–9, group 4) which differed widely in percentage of stainable pollen, when crossed with the same male parent produced progenies with relatively small differences in proportion of sterile- to fertile-pollen plants. Other female plants (80–7 and 21–2, group 5) with no significant difference in stainable pollen produced progenies having wide differences.

TABLE 6.—*Sterile-pollen and fertile-pollen plants in crossed progenies having the same male parent but different female parents*

Group	Cross	Stainable pollen in parent		Sterile-pollen plants	Fertile-pollen plants
		Female	Male		
		Percent	*Percent*	*Number*	*Number*
1	75–5 × 15–2	72.9	76.9	0	30
	80–7 × 15–2	56.4	76.9	7	9
	13–1 × 15–2	24.9	76.9	28	30
	Russet Rural × 15–2	0	76.9	60	13
2	77–9 × 75–5	[1] 5.6	72.9	6	0
	76–1 × 75–5	[1] 4.3	72.9	18	4
	77–7 × 75–5	[1] 2.8	72.9	34	3
	77–8 × 75–5	[1] 0	72.9	31	0
3	77–9 × 11–25	[1] 5.6	72.4	117	13
	76–1 × 11–25	[1] 4.3	72.4	56	1
	77–7 × 11–25	[1] 2.8	72.4	52	5
	77–8 × 11–25	[1] 0	72.4	23	1
	Russet Rural × 11–25	[1] 0	72.4	93	14
4	12–7 × 5–29	56.3	71.8	41	10
	77–9 × 5–29	[1] 5.6	71.8	58	15
	76–1 × 5–29	[1] 4.3	71.8	26	8
	77–7 × 5–29	[1] 2.8	71.8	32	3
	77–8 × 5–29	[1] 0	71.8	36	0
	Russet Rural × 5–29	[1] 0	71.8	109	4
5	15–2 × 5–1	76.9	69.5	1	55
	80–7 × 5–1	56.4	69.5	6	6
	21–2 × 5–1	55.9	69.5	43	8
	13–1 × 5–1	24.9	69.5	29	24
	Jubel × 5–1	20.9	69.5	11	14
6	77–9 × 12–7	[1] 5.6	56.3	26	11
	76–1 × 12–7	[1] 4.3	56.3	13	6
	77–7 × 12–7	[1] 2.8	56.3	68	31
	77–8 × 12–7	[1] 0	56.3	30	3

[1] Sterile-pollen plants.

BREEDING BEHAVIOR OF THE FERTILE-POLLEN PLANTS FROM A CROSS

Twenty-three fertile-pollen plants from a cross were selfed and the progenies examined to determine the proportion of sterile- to fertile-pollen plants. The study was made on a cross of 17–2×11–1 which produced a much higher proportion of sterile- to fertile-pollen plants than its reciprocal (table 3). The results obtained on the selfed and crossed progenies of the parents and on the selfed progenies of 23 F_1 fertile-pollen plants is presented in table 7. This study on the parents

TABLE 7.—*Sterile-pollen and fertile-pollen plants in the selfed, reciprocal crossed, and F_2 progenies of fertile-pollen parents 17–2 and 11–1*

Parents	Sterile-pollen plants	Fertile-pollen plants	Parents	Sterile-pollen plants	Fertile-pollen plants
	Number	*Number*		*Number*	*Number*
17–2 selfed	8	19	17–2 × 11–1–F_1–11	8	19
17–2 × 11–1	30	79	17–2 × 11–1–F_1–12	7	17
11–1 selfed	0	36	17–2 × 11–1–F_1–13	1	5
11–1 × 17–2	1	150	17–2 × 11–1–F_1–14	4	6
17–2 × 11–1–F_1–1	22	15	17–2 × 11–1–F_1–15	4	7
17–2 × 11–1–F_1–2	16	7	17–2 × 11–1–F_1–16	10	8
17–2 × 11–1–F_1–3	10	6	17–2 × 11–1–F_1–17	7	13
17–2 × 11–1–F_1–4	11	7	17–2 × 11–1–F_1–18	8	25
17–2 × 11–1–F_1–5	9	4	17–2 × 11–1–F_1–19	4	14
17–2 × 11–1–F_1–6	9	6	17–2 × 11–1–F_1–20	3	10
17–2 × 11–1–F_1–7	5	4	17–2 × 11–1–F_1–21	3	13
17–2 × 11–1–F_1–8	8	8	17–2 × 11–1–F_1–22	1	14
17–2 × 11–1–F_1–9	9	4	17–2 × 11–1–F_1–23	0	6
17–2 × 11–1–F_1–10	8	6			

was made in a later year on a portion of the same material that supplied the data in table 3. The observations do not differ significantly for the two seasons. The 23 F_1 plants studied were fertile-pollen plants taken from the reciprocal cross which had the higher proportion of sterile-pollen plants. All 23 F_1 plants produced progenies that showed segregation except 1 in which the 6 individuals were fertile-pollen plants. The range of segregation obtained indicated the presence of genetic differences between these fertile-pollen F_1 plants.

DISCUSSION

Both pollen fertility and pollen sterility have an important function in the improvement of the potato. Fertile-pollen plants are essential for the improvement of the potato through the generally accepted sexual method of breeding, while the sterile-pollen plants, being nonfruitful, will produce, other things being equal, a larger yield of tubers.

Krantz (*8*) suggests developing sterile-pollen and fertile-pollen plants with superior breeding value and then crossing sterile-pollen with fertile-pollen plants to secure nonfruittul improved varieties. The present study indicates how a breeder may proceed in order to produce improved sterile- and fertile-pollen plants. The breeder can, by choosing female parents according to their percentage of stainable pollen, obtain progenies that vary widely or progenies that approach equality in proportion of sterile-pollen to fertile-pollen plants. Female parents with 70 percent or more of stainable pollen produced progenies with a relatively high proportion of fertile-pollen plants, while female parents with little or no stainable pollen produced progenies with a

relatively low proportion of fertile-pollen plants. The female parents having between 10 and 70 percent of stainable pollen produced progenies with sterile-pollen and fertile-pollen plants in proportions that would be suitable for the selection of superior plants of both types. In the 70-percent group, a higher accuracy of estimate of breeding behavior as female parents may be obtained by supplementing th information on stainable pollen with that on breeding behavior, when selfed. Attention should be called to the apparent absence of association between both the percentage of stainable pollen in a plant and its breeding behavior as a female parent with its breeding behavior as a male parent. Significant and consistent differences between plants when used as male parents were observed. Thus the known breeding behavior of a plant when used as a male parent may prove to be a further aid to the breeder in producing the type of progenies in respect to pollen sterility that he desires.

SUMMARY

The problem presented in potato breeding by the anomalous relation of pollen sterility to efficient breeding procedure and to tuber yield was studied.

The breeding material was classified as sterile-pollen and fertile-pollen plants, and the latter were further classified according to their percentage of pollen grains stainable with acetocarmine.

Wide differences were found in the proportion of sterile- to fertile-pollen plants in five sets of reciprocal crosses. Each reciprocal set was made between two parents having significantly different percentages of stainable pollen. In each set the combination low × high percent stainable pollen gave a low proportion and the high × low a high proportion of fertile-pollen plants.

In 5 of nine plants tested the selfed progeny was similar in the proportion of sterile- to fertile-pollen plants to the crossed progeny when the plant was used as a female parent. The significant differences found between the two types of progenies for 2 of the plants indicated that in some combinations the pollen parent influenced the proportion of sterile- to fertile-pollen plants in the progeny.

A study of eight groups of crosses with the progenies within each group having the same female parent showed that progenies with the same female parent but with different male parents may be significantly different in the proportion of sterile- to fertile-pollen plants. This difference was neither related to the percentage of stainable pollen in the male parents nor to their breeding behavior when selfed.

A similar study of seven groups of crosses with the progenies within each group having a common male parent showed that progenies having a common male parent but different female parents may differ widely in the proportion of sterile-pollen to fertile-pollen plants. This difference was associated with the percentage of stainable pollen in the female parent and its breeding behavior when selfed.

Twenty-three fertile-pollen F_1 plants from a cross of low × high percent stainable pollen produced selfed progenies of which all except one segregated for sterile-pollen and fertile-pollen plants. This one produced six plants all of which were fertile-pollen plants.

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STUDIES ON BIOLOGICAL RACES OF THE HESSIAN FLY[1]

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INTRODUCTION

With the increasing interest and progress in breeding wheats resistant to the hessian fly (*Phytophaga destructor* (Say)), the authors feel that the information they have obtained since 1935 on biological races of the fly, i. e., races differing greatly in their ability to infest different varieties of wheat through apparently identical taxonomically, may be of timely value. This information supports the earlier conclusions of Painter (*6*).[2]

ISOLATED GEOGRAPHICAL POPULATIONS OF THE FLY

The hypothesis of biological races of the hessian fly has been offered to explain the contradictory results in fly-resistance tests on wheat varieties in regions of the United States that are separated by physical barriers or distinguished by climatic differences. A striking variability of reaction occurs, for instance, in the variety Dawson, which is resistant in California (*5*) and Kansas (*4*), but susceptible in Illinois and Indiana.[3]

When the testing program on wheat varieties was expanded in 1936 in California, a nursery of 275 American wheat varieties and strains was seeded and exposed to a severe attack of the hessian fly in the field at Birds Landing, Calif. Thirty varieties, including China, Clarkan, Dawson, Dixon, Emerald, Goens, Huston, Java, Marquillo, Marvel, Nabob, Red Rock, Shepherd, Mediterranean, and others, showed high resistance to the fly.

These resistant varieties were retested at La Fayette, Ind., in 1936 in the field, and the complete series of classified wheats (*3*), except for a few omissions, was tested in the greenhouse during the period from 1938 to 1940, inclusive. With the exception of Dixon, Java, Marquillo, and Marvel, the common varieties of wheats were all susceptible to the general population of Indiana hessian flies. Although these results were explainable on the basis of a difference in ability of the California and Indiana populations of the fly to infest the wheats, the possibility that they were due to variation in the wheats themselves in response to environmental differences between the two regions was not eliminated. A small number of resistant and susceptible wheat varieties was therefore tested under uniform environmental conditions in the greenhouse

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[2] Italic numbers in parentheses refer to Literature Cited, p. 153.

[3] Unpublished data on several tests during the period 1920–44.

during 1936, 1939, and 1940 at La Fayette, Ind., flies emerging from flaxseeds collected at Birds Landing, Calif., and at La Fayette being used.

The wheats were infested in the young seedling stage under cages according to a method in use at the La Fayette laboratory (*1*). That there were differences in the reactions of the respective varieties to the flies from the two regions is apparent from the data presented in table 1. The records there shown are in agreement with those of the earlier field tests, and this fact strengthens or confirms the hypothesis that there are distinct regional races of the hessian fly in California and Indiana.

TABLE 1.—*Extent of infestation by hessian flies from California and Indiana in wheat varieties grown in the greenhouse at La Fayette, Ind., 1936 and 1939–40*

Variety of wheat	California flies					Indiana flies				
	1936	1939	1940A	1940B	Average	1936	1939	1940A	1940B	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dawson	0	11	5	10	6.5	89	89	90	100	92.0
Ill. No. 1 W38–6	16	11	14	10	12.7	5	63	15	25	27.0
Marquillo	------	30	35	33	35.7	------	31	35	40	35.3
Wabash	14	31	43	32	30.0	87	84	95	95	90.2
Big Club	80	100	100	95	93.7	100	95	95	100	97.5

HOST-RESTRICTED RACES

Tests reported by Painter (*6*) and Painter, Salmon, and Parker (*7*) showed that there were differences in the capacity of individual flies within a single population to infest different varieties of wheat in the greenhouse. Such findings are highly important and more significant than the occurrence of geographical races of the hessian fly. If a given population in a locality is composed of interbreeding individuals differing with respect to their ability to infest different wheat varieties, the planting of a variety resistant to most but not all individuals in the fly population may result in the natural selection of a local race or population of flies that will be capable of heavily infesting the erstwhile resistant wheat. In view of the importance of obtaining more information on this possibility, efforts to determine whether such a race can be isolated experimentally were made at La Fayette.

The genetic features of the problem resemble those encountered in the study of cereal smut and rust fungi, and a similar technique of investigation is adaptable to their study. Adult hessian flies reared from the few puparia found in resistant wheat were used as the parents of possible host-restricted races. The flies bred and used experimentally during the period 1936–42 did not increase or survive beyond the second generation on resistant wheats. In 1943, however, a selected population which showed a greater ability than that of the general population to mature on Java, Dixon, Marvel, and Ill. No. 1 W38 was obtained in the course of routine tests in the greenhouse from a series of resistant American and foreign wheats including both common and durum varieties. This population was bred and tested in March 1943 and during the period from October 1943 to March

1944. In the tests reported herein this selected population of the fly was compared with the general fly population which had been used continuously for experiments in the greenhouse for 2 years and, because of continuous inbreeding and use on resistant wheats, may have differed from a strictly wild population. It is certain that the general population had within its composition some individuals genetically similar to the ones comprising the selected population, inasmuch as it was the source of the selected population, but logically such individuals would be present in smaller numbers. In all these tests eggs were permitted to be laid on the plants in such numbers as to eliminate the possibility that lack of oviposition on the part of the flies representing either the selected or the general population would account for differences in final infestation.

The first generation of the selected population of the hessian fly was tested on 5 resistant wheats in the greenhouse in March 1943. Four 1-row plantings of young wheat seedlings of each variety, each containing about 20 plants, were exposed to 400 flies of the selected population, and at the same time 4 similar plantings were exposed to 400 flies of the general population. As shown in table 2, the percent-

TABLE 2.—*Extent of infestation by hessian flies from selected and general populations in wheat varieties grown in the greenhouse at La Fayette, Ind., March 1943*

Variety	Flies from selected population				Flies from general population			
	Plants examined	Plants infested	Plants stunted [1]	Puparia	Plants examined	Plants infested	Plants stunted [1]	Puparia
	Number	*Percent*	*Percent*	*Number*	*Number*	*Percent*	*Percent*	*Number*
Ill. No. 1 W38-6-11	89	44	24	89	78	5	1	7
IVy	83	34	20	93	83	20	4	50
P. I. 111245-10	80	23	14	36	73	8	7	16
P. I. 56206-8-7	110	7	7	25	109	4	3	5
P. I. 94587	98	0	0	0	76	0	0	0
Michigan Amber (susceptible check)	80	100	100	551	80	100	100	671

[1] Stunted by infestation.

age of plants stunted by infestation (plants reacting as susceptible), the percentage of plants infested including those stunted, and the total puparia were significantly greater for the selected population with the exception of those on the highly resistant durum wheat P. I. 94587.

The second generation was reared as stock material in October and November 1943, on the fly-resistant wheats Ill. No. 1 W38, B36162A13–12, and A3848A5–5. The number of individual flies emerging after the storage of material during the summer was too small for tests in this generation. The wheat lines B36162 and A3848 mentioned above are soft, winter-type segregates derived from the backcrossing of Ill. No. 1 W38 with soft red winter wheats in the cooperative project for breeding wheats resistant to the hessian fly at the Purdue University Agricultural Experiment Station.

The third, fourth, fifth, and sixth generations of the selected population of the hessian fly came from rearings through the fly-resistant

wheats B36162A13–12 and A3848A5–5 in the greenhouse during the period December 1943 to March 1944, inclusive. With each successive generation through the resistant wheats, the infestations of B36162A13–12 and A3848A5–5 and of the susceptible check Wabash by the selected and general populations were recorded. In each test 50 female flies selected at random from the respective populations were used to infest the individual plantings, which consisted

FIGURE 1.—Wheat strains exposed to selected and general populations of the hessian fly: Rows *a* to *c* exposed to selected population of the fly, and rows *d* to *f* to the general population. Rows *a* and *f*, Wabash (susceptible); rows *b* and *e*, B36162A13–12 (resistant); and *c* and *d*, A3848A5–5 (resistant).

of approximately 45 plants of each of the three wheats. A summary of all tests is given in table 3.

TABLE 3.—*Extent of infestation by selected and general populations of hessian flies in B36162A13–12, A3848A5–5, and Wabash wheats grown in the greenhouse at La Fayette, Ind., December 1943 to March 1944*

Variety	Total tests	Flies from selected population		Flies from general population	
		Infested plants	Stunted plants [1]	Infested plants	Stunted plants [1]
	Number	*Percent*	*Percent*	*Percent*	*Percent*
B36162A13–12	33	73.5	62.6	43.3	33.5
A3848A5–5	32	67.6	58.8	36.6	27.5
Wabash	33	100	100	100	100

[1] Stunted by infestation.

Table 3 shows that the percentage of susceptible or stunted plants in the resistant varieties infested with the selected population of the

fly was approximately double that of plants infested with flies from the general population. There were highly significant visual differences in the reactions of the two populations (fig. 1) which were apparent in most tests.

Infestations of 80 percent or more of the plants by the selected population were not uncommon in the tests, but infestations varied greatly among the individual tests. This variation indicates that the selected population was not homozygous after six generations of screening through the resistant wheats. The results appear to demonstrate quite conclusively, however, that a strain or race of the hessian fly capable of maturing successfully in and seriously injuring wheats that are highly resistant to the general fly population can be segregated from that population.

TABLE 4.—*Extent of infestation by hessian flies from the fourth-generation selected population in wheat varieties grown in the greenhouse at La Fayette, Ind., March 1944*

Variety [1]	Plants examined	Plants infested	Plants stunted [2]
Common wheat:	*Number*	*Percent*	*Percent*
Wabash, check	249	100	100
B36162A13-12, check	202	98	96
Alberta Early, C. I. 10025–2	28	100	100
Centenario, Ks. 38 F. N. 4002	27	96	96
Dixon, C. I. 6049	30	93	93
Greek 10, P. I. 116227	30	100	100
IVcl, Ks. 36 R. N. 3579	25	96	96
IVy, Ks. 36 R. N. 3580	24	88	88
Java, C. I. 10051	35	97	97
Marquillo, C. I. 6887	27	37	37
Marvel, C. I. 8876	26	100	100
Portuguez, P. I. 56204–7	30	13	13
Ribeiro, P. I. 56206–8	27	0	0
Triunfo, P. I. 104138	22	100	100
Unnamed, P. I. 94549–6	29	14	14
Unnamed, P. I. 94571–14	32	9	6
Unnamed, P. I. 111245–10	32	100	100
Durum wheat:			
Tremez rijo, P. I. 56257–1	25	0	0
Unnamed, P. I. 94587	42	0	0
Emmer:			
Yaroslav, C. I. 1562	62	0	0

[1] C. I. after varietal name refers to accession number of the Division of Cereal Crops and Diseases and P. I. to accession number of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, and Ks. to the accession number of the Kansas Agricultural Experiment Station.

[2] Stunted by infestation.

Seventy-eight wheat varieties and strains resistant to the general population of the hessian fly (*2*) were tested with the selected population of the fourth generation in March 1944. Many adult flies emerging from the stocks of the selected population were permitted to oviposit heavily on the wheat seedlings, thereby increasing the chances of infestation by individuals having the ability to mature in them. The percentage of stunted or susceptible plants resulting in most of the common varieties approached that of the susceptible check, but the common wheat variety Ribeiro, the highly resistant durums, and the emmer variety Yaroslav were uninfested. A partial list of the varieties and their reaction to the selected population is

given in table 4 to show the variation in infestation among those included in this test. In this series a check of the plants of B36162A13–12 showed 41 percent stunted by the general population of the fly in contrast to the 96 percent for the selected population, which indicated that the expression of susceptibility in the resistant wheats was not due to adverse environmental conditions in the greenhouse at the time of the tests. The probability of further segregation of specialized strains of flies through host restriction of populations is indicated by the differences in infestations occurring in such common varieties as Marquillo C. I. 6887, Ribeiro P. I. 56206–8, and unnamed P. I. 94549–6.

The results of the several tests reported in this paper indicate that a fully effective breeding program for the development of fly-resistant commercial hybrids or varieties of wheat must provide for the possible appearance of biological races of the hessian fly within regions as well as for their present known occurrence in different regions. This involves not only the study of genetically different factors for hessian fly resistance in wheat varieties, but also a study of the genetic diversity of fly populations.

SUMMARY

The tests at La Fayette, Ind., since 1935 have substantiated the existence of different biological races of the hessian fly in California and Indiana indicated in previous studies by the writers and others. The characteristics of the two populations as noted in field trials in the two regions were maintained when tested on resistant and susceptible wheats in the greenhouse at La Fayette, Ind. Varieties such as Dawson and Wabash, which were resistant to the California populations of the fly, were susceptible to the Indiana population.

In the studies on host-restricted races of the hessian fly, a population was bred from the general population at La Fayette which was capable of heavily infesting many resistant common wheats, such as Dixon, Java, and Marvel. This selected population was bred and tested for six generations on resistant wheats, including the winter lines derived from crosses with Ill. No. 1 W38. The percentage of plants stunted or susceptible in the resistant varieties was doubled after breeding the selected population through the third to sixth generations, but it did not attain the 100 percent injury of susceptible wheats.

The selected population of the hessian fly was cultured for more extensive tests on resistant wheats, and the differences in infestations obtained in varieties such as Marquillo, Ribeiro, and other varieties indicated the probability of further segregation of the selected population. Several durum wheats which appeared to be immune to the general population of the hessian fly were uninfested by the selected population.

The results indicate that a fully effective program for breeding resistance to the hessian fly must provide for the possible appearance of biological races of the fly within regions as well as for their known presence in different regions, through a study of the genetic diversity of fly populations as well as a study of the genetically different factors for hessian fly resistance in different varieties of wheat.

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VALUE OF SOYBEAN MEAL PREPARED FROM FROSTED-FIELD-DAMAGED SOYBEANS FOR GROWING-FATTENING SWINE [1]

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INTRODUCTION

During the fall of 1942 large areas of soybeans in the central part of the Corn Belt were frosted before maturity, and the quality of the beans was further reduced by unfavorable weather which postponed harvest until late winter or early spring. Such beans are referred to in this paper as frosted-field-damaged soybeans.

Soybean meal was prepared by the expeller process from a sample of these frosted-field-damaged soybeans for comparison with expeller soybean meal prepared from sound mature beans of the 1942 crop. The damaged soybeans, which were harvested in 1943, are thus described by T. H. Allwein: [3]

> These beans were just straight country run beans, which were harvested from the fields during March. These beans were of mixed varieties, which originated in this locality [Gibson City, Ill.]. All of these beans were sample grade beans and averaged about 40 percent damage and 48 pounds test weight. There also was considerable dockage, on an average of about 3 percent. This meal was produced by our regular expeller process. . . .

METHODS

In order to obtain quantitative results the paired-feeding method for equal gains was used. One pig of each pair was fed the check ration which contained normal soybean meal, while its pair mate was fed the mixture which contained soybean meal prepared from the frosted-field-damaged soybeans.

Sixteen Poland China pigs which had been on rye and alfalfa pastures during the spring and early summer were paired on the basis of weight, litter, sex, type, condition, and probable outcome. All pigs were thrifty.

The basal mixture used consisted of ground yellow corn, soybean meal, alfalfa meal, and a mineral mixture. The proportion of corn

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[2] The writers gratefully acknowledge the assistance of R. H. McDade, chief swine herdsman.

[3] Personal correspondence. The damaged soybean meal was prepared and donated to the University of Illinois by the Central Soya Co., Inc., Gibson City, Ill.

and soybean meal in the mixture was varied in order to change the percentage of protein in the ration as the pigs increased in weight. The percentages fed are given in table 1. The alfalfa meal was fed at the 10-percent level during the test period. The generous feeding of alfalfa meal was to insure against a possible vitamin deficiency in the all-vegetable ration, as discussed by Krider, Fairbanks, and Carroll.[4]

TABLE 1.—*Variations in the proportion of the ingredients of the rations fed to growing swine at different live weights*

Feeds	Proportion in which feeds were mixed for pigs weighing—					
	75 pounds or less		75 to 150 pounds		Over 150 pounds	
	Check ration	Test ration	Check ration	Test ration	Check ration	Test ration
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Ground yellow corn	53.0	53.5	65.0	65.5	71.0	71.0
Normal soybean meal	34.5		22.5		16.5	
Damaged soybean meal		34.0		22.0		16.5
Alfalfa meal	10.0	10.0	10.0	10.0	10.0	10.0
Ground limestone	.5	.5	.5	.5	.5	.5
Steamed bonemeal	1.5	1.5	1.5	1.5	1.5	1.5
Iodized salt	.5	.5	.5	.5	.5	.5
Total	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein (percent)	21.0		17.0		15.0	

The yellow corn, which graded No. 2, was estimated to contain 9.0 percent crude protein. Analysis of the alfalfa meal showed 14.6 percent crude protein. The chemical composition of the soybean meals is given in table 2.

In preparing the feed mixtures, the slightly higher protein content of the damaged soybean meal was taken into consideration by equalizing the total percentage of crude protein in the mixtures for the pigs in each weight-group interval. The percentages of crude protein fed are given in table 1.

The pigs were fed twice daily in individual feeding crates. The feed allowances were weighed to one-tenth of a pound and fed in a metal trough with which each crate was equipped. A small amount of water was poured on the feed to prevent waste. The pigs were closely observed in an attempt to keep feed consumption up to the limit of the gains of the slower gaining pig of each pair. Feed refusals were noted. Individual weights of all pigs were taken at weekly intervals and the feed allowances for the following week were adjusted in accordance with the gains made during the previous week. This method was considered satisfactory, as reported previously by Krider, Fairbanks, and Carroll.[4]

[4] KRIDER, J. L., FAIRBANKS, B. W., and CARROLL, W. E. VALUE OF SOYBEAN MEAL PREPARED FROM DAMAGED (BIN-BURNED) SOYBEANS AS A FEED FOR GROWING SWINE. Jour. Agr. Res. 69: 383–387. 1944.

TABLE 2.—*Chemical composition of the soybean meals on the fresh basis*

Feeds	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Normal soybean meal	88.76	42.75	4.65	5.14	4.58	31.64
Damaged soybean meal	88.41	43.25	4.79	5.39	4.45	30.53

The eight pairs of pigs were kept on concrete floors, in one group, and had access to an outside concrete runway.

The experiment began on July 13, 1943, and continued until both pigs of each pair had attained a final weight of approximately 200 pounds.

RESULTS AND DISCUSSION

The results of the experiment are summarized in table 3. The table shows that in five of the eight pairs the check pig required more feed per pound of gain, while in three pairs the test pig required more. These results on economy of gain are too nearly a chance distribution to indicate any significant differences in the feeding value of the two mixtures. This conclusion was verified by statistical treatment of the data by Students' method with argument t for paired differences as described by Snedecor.[5]

The record of feed refusals indicated that the rations were equally palatable, and the χ^2 test showed that the difference in feed refusals was largely a chance deviation.

To obtain ideal results in paired feeding for equal gains, the pigs of each pair should make the same weekly gain in live weight. However, this rarely happens. With the 8 pairs of pigs used in these experiments, there was a total of 177 weekly comparisons between the gains of pair mates. Of the 177 comparisons, only 17 showed identical gains, while in 82 the check pigs gained slightly more and in 78 the test pigs gained more. The deviation of 2 from the expected, assuming a chance distribution, is not significant, which indicates that the method of feeding for equal gains used in the present work was quite successful.

The average daily gains are probably smaller than the feed mixtures are capable of supporting under conditions of unrestricted feeding, but this is to be expected when either gain or feed intake is controlled in paired feeding.

It may be observed in table 3 that the average daily feed was greater for the check pig in 5 pairs and greater for the test pig in 3 pairs. From an analysis of the 177 weekly comparisons of the feed intake of pair mates, it was found that slightly more of the check ration was required to produce the same gains as the ration which contained damaged soybean meal. In 19 of these comparisons, pair mates consumed the same amount of concentrates, in 98 the check pigs consumed more, and in 60 the test pigs consumed more. The χ^2 test indicates that the deviation of 19 from the ideal of a chance

[5] SNEDECOR, G. W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. 1940. Ames, Iowa.

TABLE 3.—*Weights, gains, and feed consumption of 8 pairs of pigs, one of each pair being on the check ration and the other on the damaged soybean-meal (test) ration*

Item	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7		Pair 8		Average	
	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig
Final weight.....pounds	205	200	205	208	214	212	197	202	198	198	202	200	207	201	205	202	204.1	202.9
Initial weight.....do	66	60	50	58	62	62	49	48	45	47	70	72	74	65	60	59	59.5	58.9
Total gain.....do	139	140	155	150	152	150	148	154	153	151	132	128	133	136	145	143	144.6	144.0
Period on test.....days	147	147	147	147	147	147	182	182	154	154	154	154	147	147	161	161	155	155
Average daily gain.....pounds	.95	.95	1.05	1.02	1.03	1.02	.81	.85	.99	.98	.86	.83	.90	.92	.90	.89	.94	.94
Total feed eaten.....do	524.4	493.4	530.9	490.6	429.9	525.3	579.8	537.7	504.5	485.3	466.6	487.2	537.4	502.1	500.5	505.8	517.1	503.4
Average ration.....do	3.57	3.36	3.61	3.34	3.35	3.57	3.19	2.95	3.28	3.15	3.03	3.16	3.66	3.42	3.11	3.14	3.35	3.26
Feed consumed per pound of gain...do	3.77	3.52	3.43	3.27	3.24	3.50	3.92	3.49	3.30	3.21	3.53	3.81	4.04	3.69	3.45	3.54	3.58	3.50

distribution was not due to chance alone, and that some other factor or factors were probably operating. The χ^2 value of 9.14 is significant. The authors believe that the values for feed per 100 pounds of gain are more important criteria than the comparison of weekly feed intake and hence that little or no significance should be attached to the comparisons of the weekly feed intakes.

Within the limits of error of the method, the data indicate that the two meals have practically the same energy value. Since the levels at which protein was fed were not border line but were considered optimum, it cannot be concluded that protein in the meal from the frosted-field-damaged soybean is as good for promoting growth as that in the meal from the normal soybean. At the protein levels fed, however, the two meals were equally efficient in supplementing corn, alfalfa meal, and minerals for growing-fattening pigs fed in dry lot under the conditions of this experiment.

The inclusion of 10 percent of alfalfa meal in the rations may possibly have obscured differences in the heat-labile constituents, such as some of the vitamins, contained in the two soybean meals. The purpose of adding the alfalfa meal was to prevent the vitamin deficiencies which occur when a ration containing corn, soybean meal, and minerals is fed to pigs in dry lot.

SUMMARY AND CONCLUSIONS

In the central Corn Belt large areas of soybeans of the 1942 crop were frosted before maturity and were further damaged by unfavorable weather which prevented harvest until the spring of 1943. Some of these frosted-field-damaged soybeans were described as Sample Grade mixed soybeans, 40 percent damaged, with a test weight of 48 pounds per bushel. Soybean meal prepared by the expeller process from these damaged soybeans was compared with soybean meal, prepared by the same method, from sound soybeans in feeding experiments with growing-fattening pigs.

The method of paired feeding for equal gains was used in this test, which involved eight pairs of pigs fed in dry lot. The soybean meals supplemented feed mixtures composed of ground yellow corn, alfalfa meal, steamed bonemeal, ground limestone, and iodized salt. In three pairs of pigs, the checkmates made more economical gains than the pigs fed the soybean meal from frosted-field-damaged beans, while in five pairs the economy of gains favored the latter pigs. These results were studied statistically and the differences were found to be statistically insignificant.

It is concluded (1) that the energy value of the two meals is the same for growing-fattening pigs, and (2) also at the protein levels fed, the two meals were equally efficient in supplementing corn, alfalfa meal, and minerals

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